

Rule-based Modeling of Signal Transduction

Jim Faeder

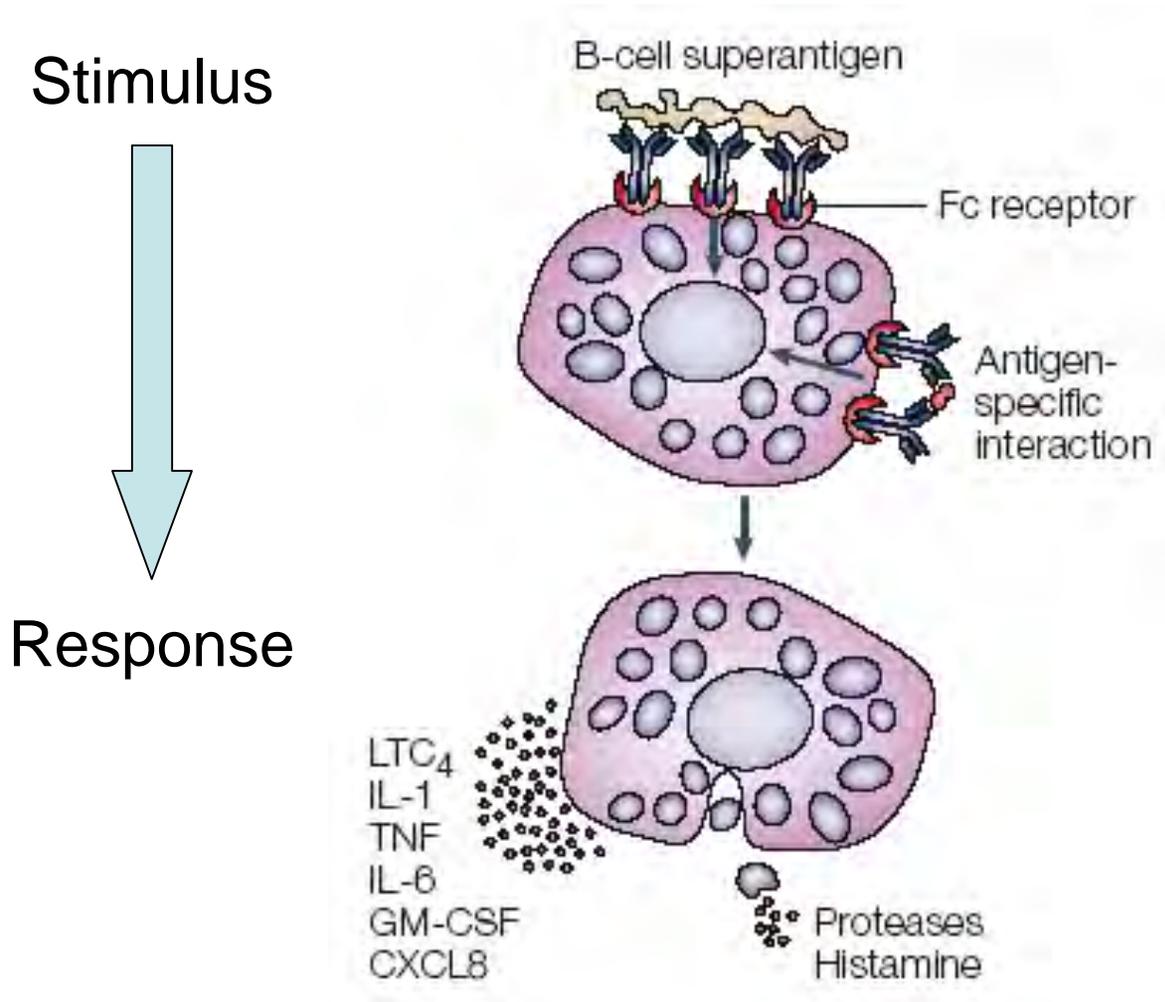
*Theoretical Biology and Biophysics Group (T-10)
Los Alamos National Laboratory*

**q-bio Summer School
July 25, 2007**

Cell signaling - *cellular information processing* - is critical to the survival of all organisms and plays a critical role in human health and disease.

Our goal is to develop predictive models of cell signaling to better understand and control these processes.

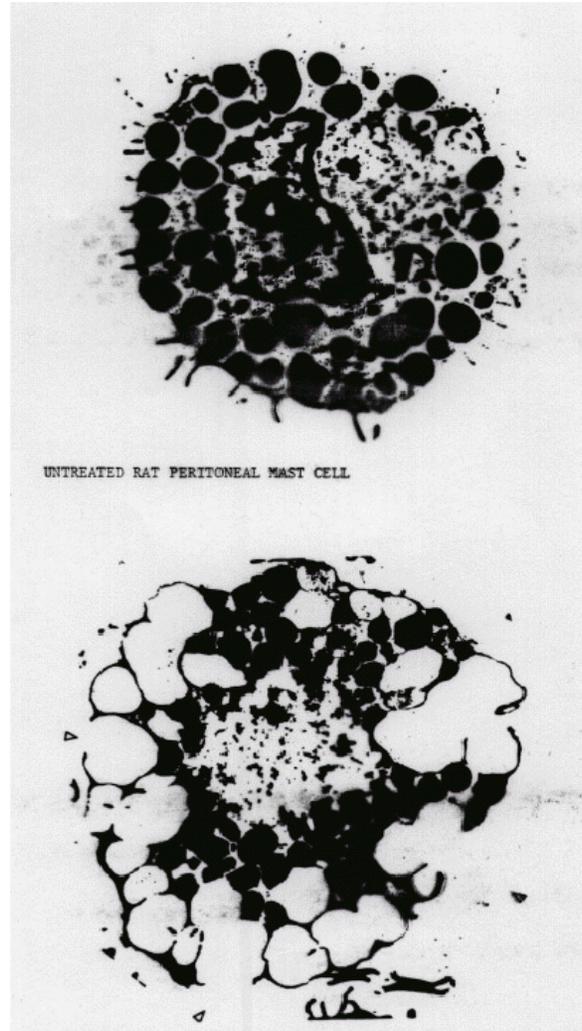
Cell signaling in allergic responses



Marshall, *Nat. Rev. Immunol.* (2004)

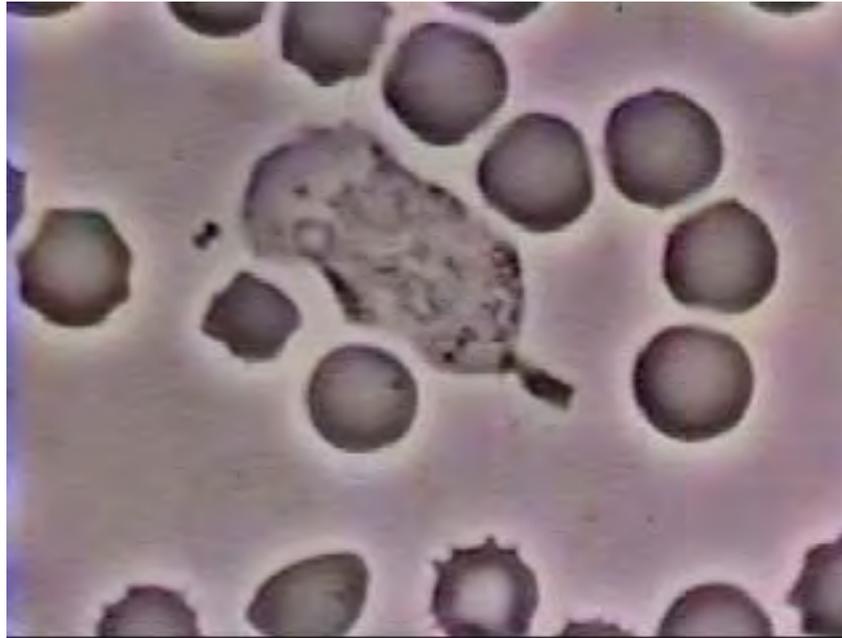
Mast cell “degranulation” is a critical component of many allergic responses

Mast cell at rest



**3 min. after
exposure to
allergen**

Movie: An immune cell in action



Original film from David Rogers (Vanderbilt University)

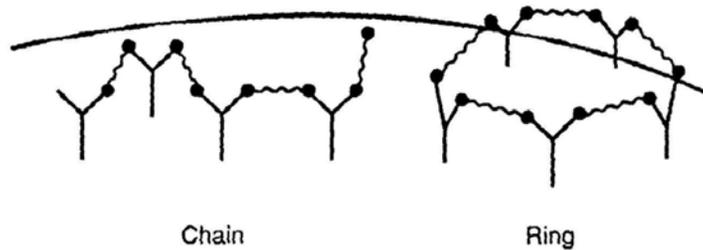
http://www.biochemweb.org/fenteany/research/cell_migration/neutrophil.html

Goals

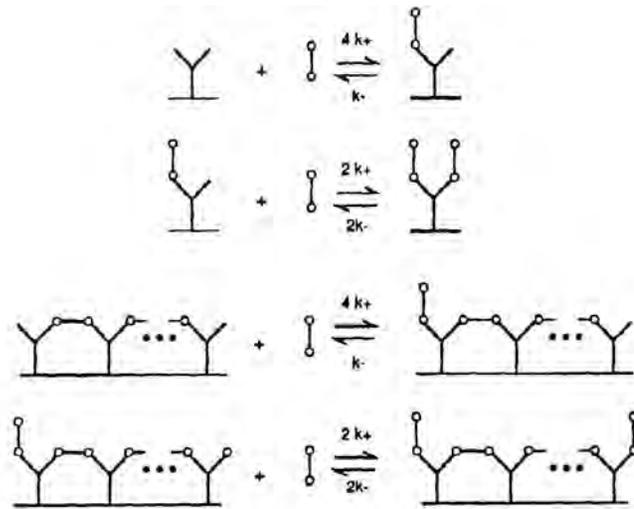
- Predictive understanding
 - Different stimulation conditions
 - Protein expression levels
 - Manipulation of protein modules
 - Site-specific inhibitors
- Mechanistic insights
 - Why do signal proteins contain so many diverse elements?
- Drug development
 - New targets
 - Combination therapies

Los Alamos approach to modeling: The past (70s-90s)

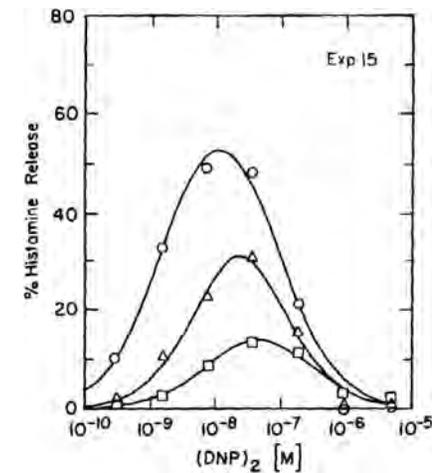
1. Multivalent binding process



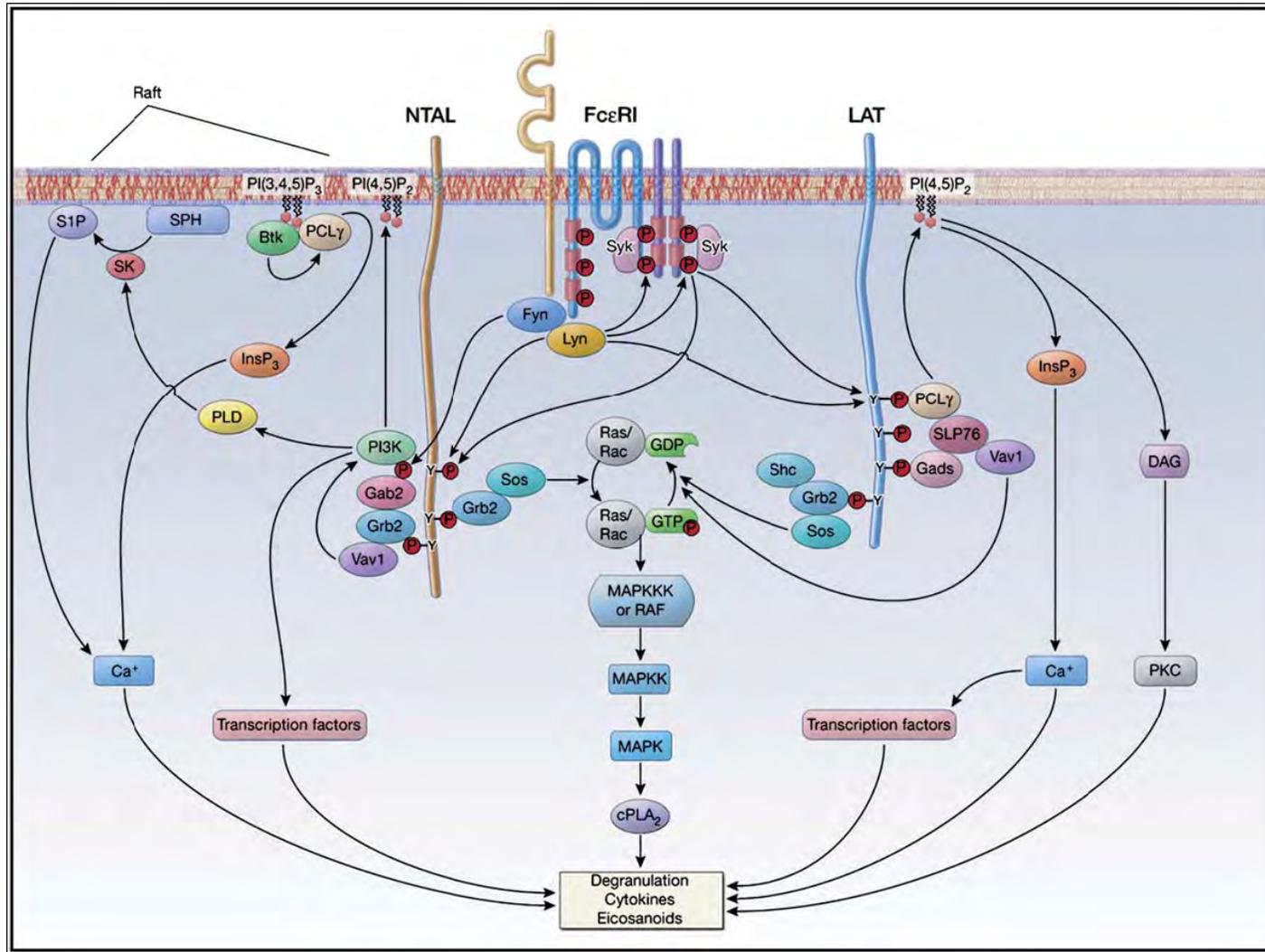
2. Rules that define network



3. Quantitative predictions

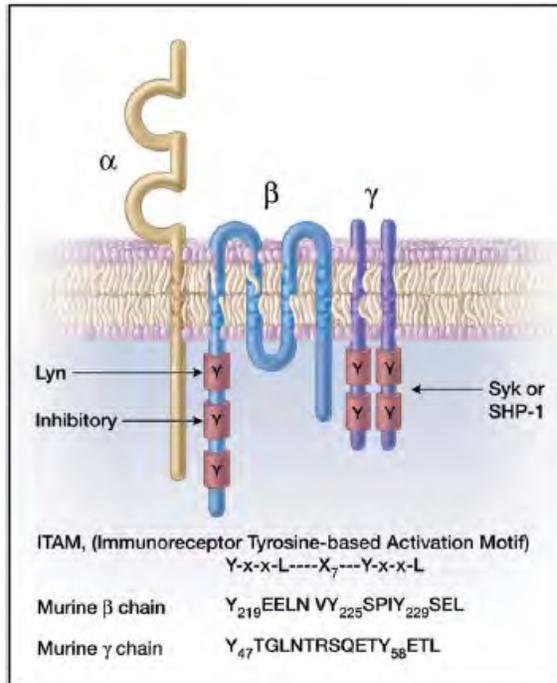


Toward models of intracellular signaling



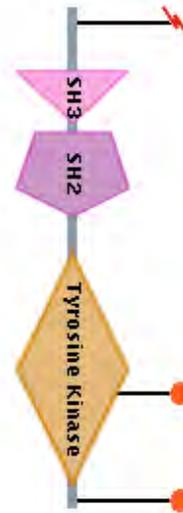
J. Rivera and A. Gilfillan, *J. Allergy Clin. Immunol.* **117**, 1216 (2006).

Modularity of signaling proteins

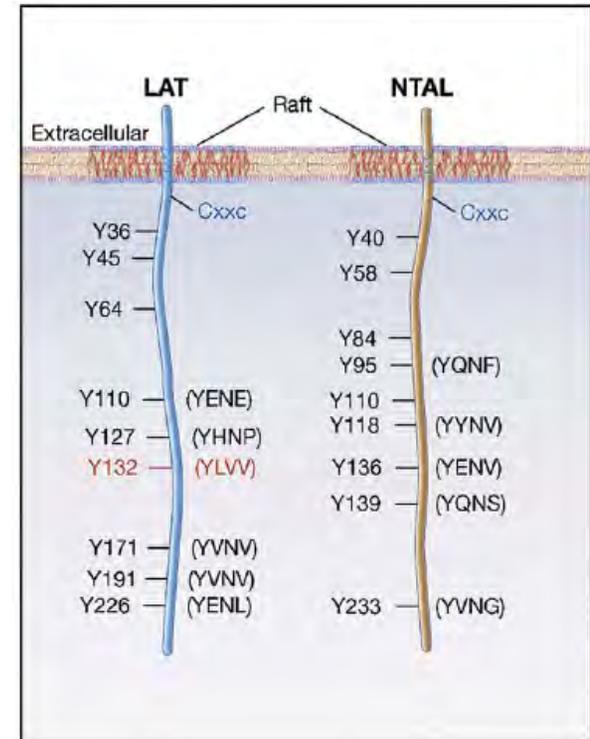
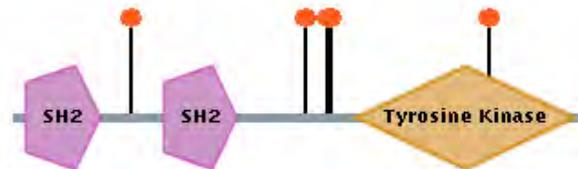


FcεRI

Lyn

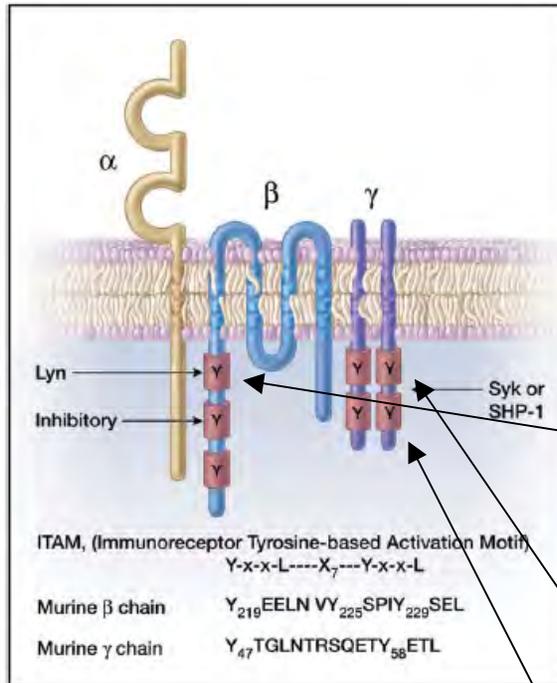


Syk



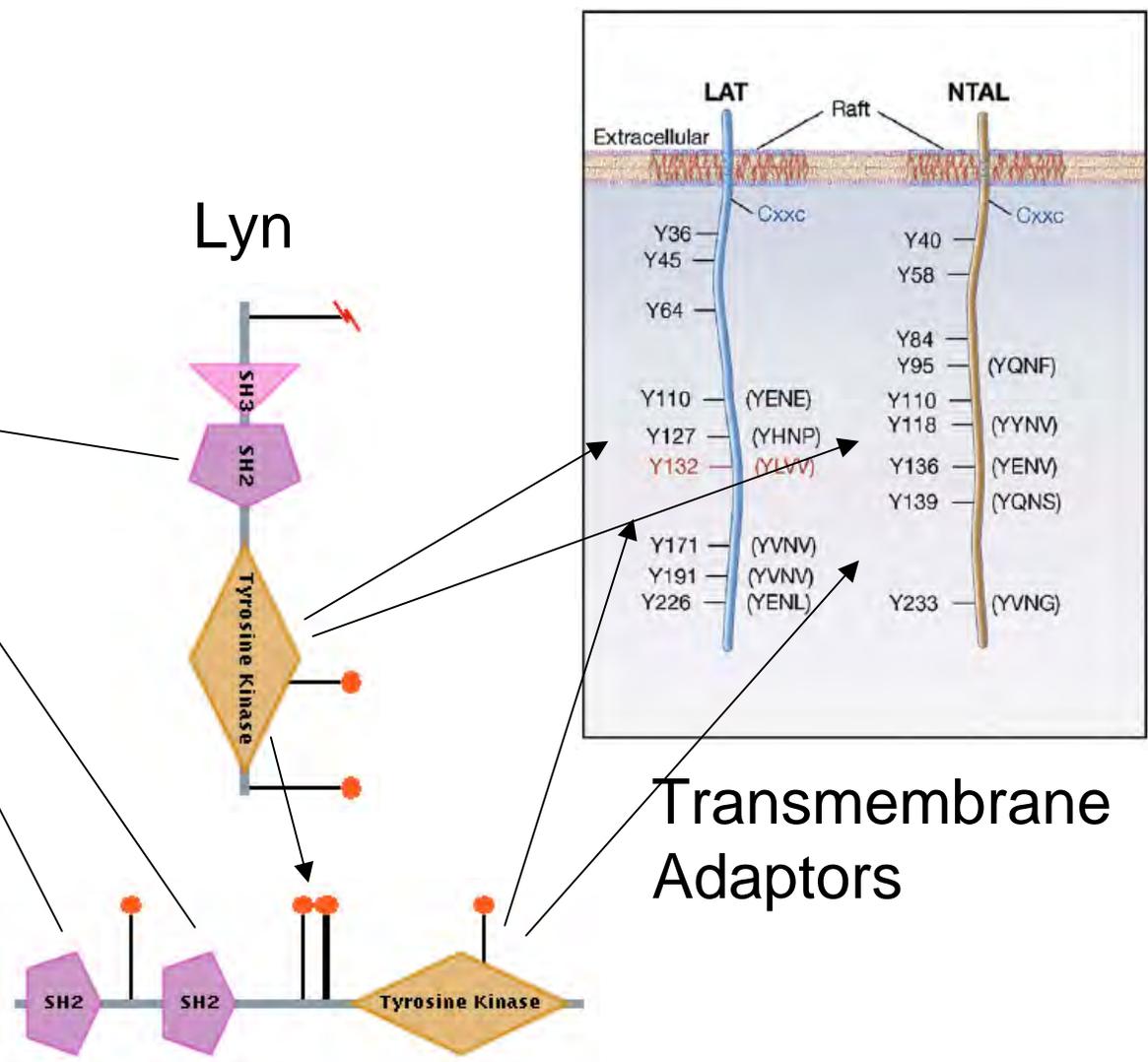
Transmembrane Adaptors

Signaling proteins contain domains and motifs that mediate interactions with other proteins



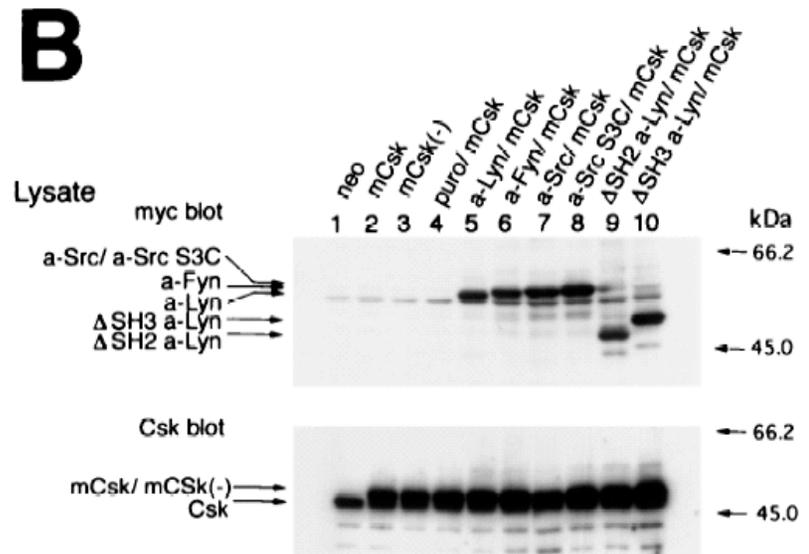
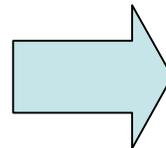
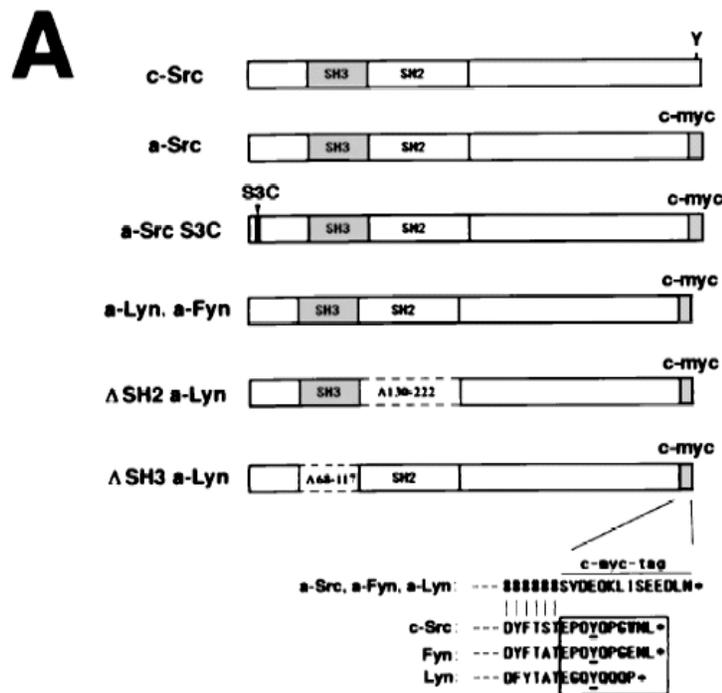
FcεRI

Syk



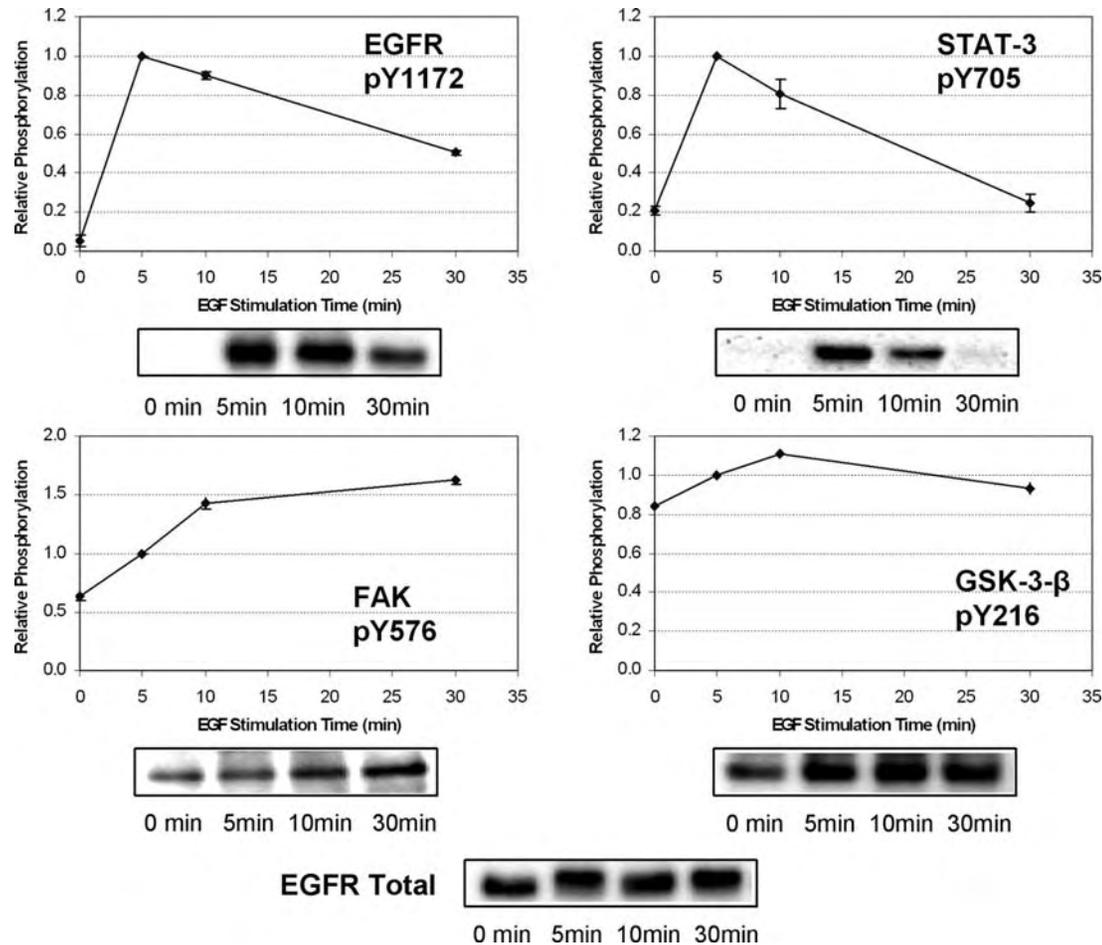
Experiments probe the function of protein modules

These modules may mediate both protein-protein and protein-lipid interactions



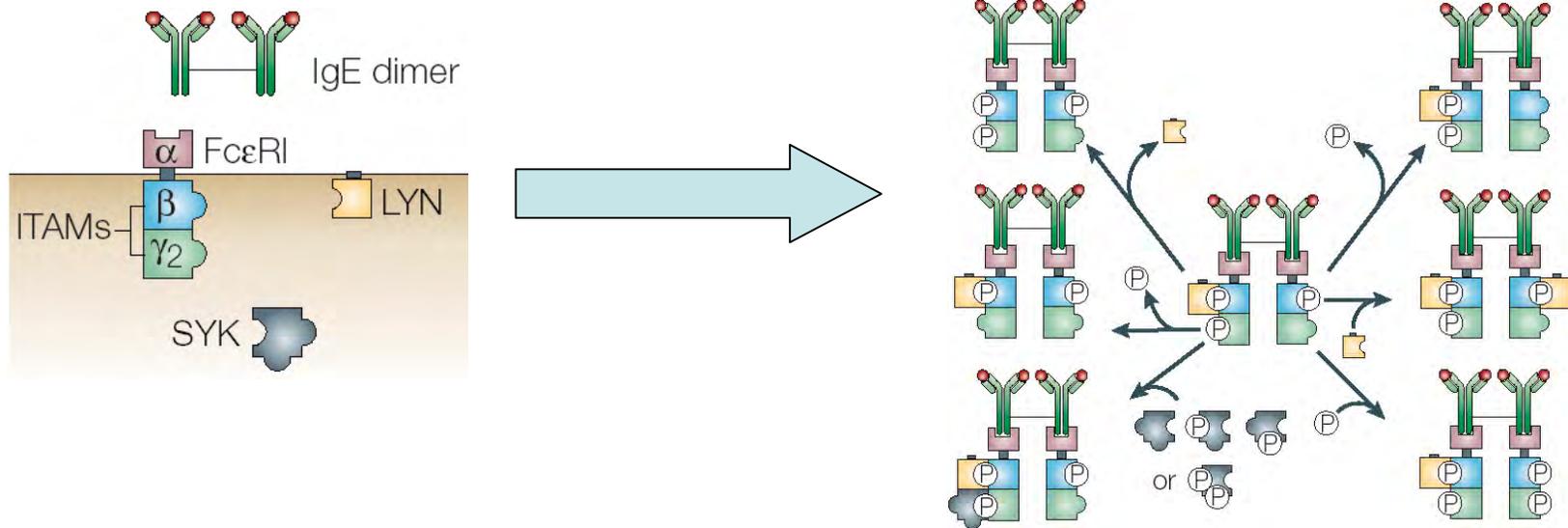
Honda *et al.*, Mol. Cell. Biol. (2000), **20**, 1759.

Experiments probe the kinetics of multiple phosphorylation sites



Zhang et al., *Mol. Cell. Proteomics* 4, 1240 (2005).

Early IgE receptor signaling exhibits combinatorial complexity

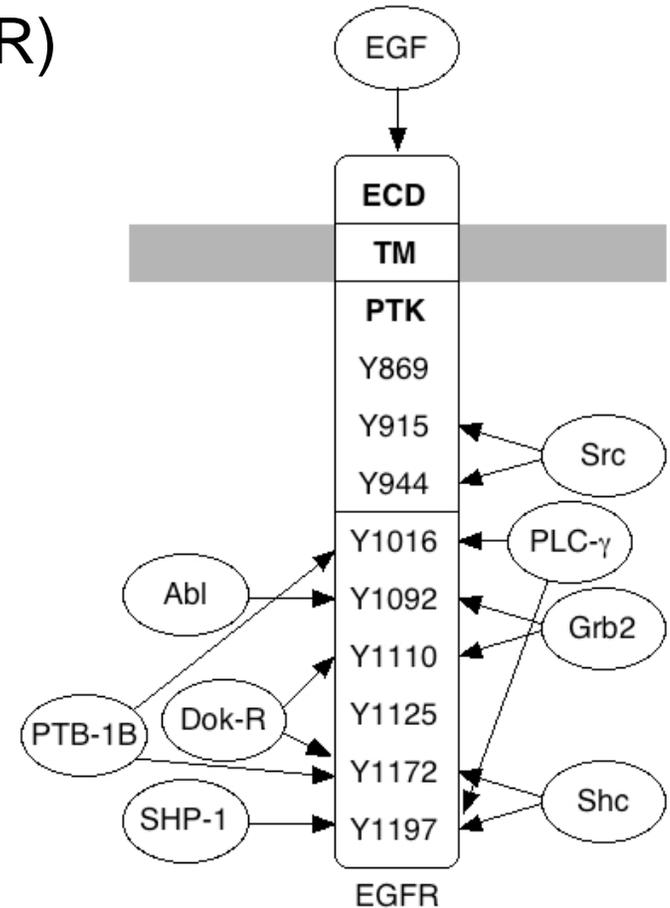


354 species / 3680 reactions

Combinatorial complexity = small number of components and interactions gives rise to a large network of species and reactions

Multiplicity of sites and binding partners gives rise to combinatorial complexity

Epidermal growth factor receptor (EGFR)



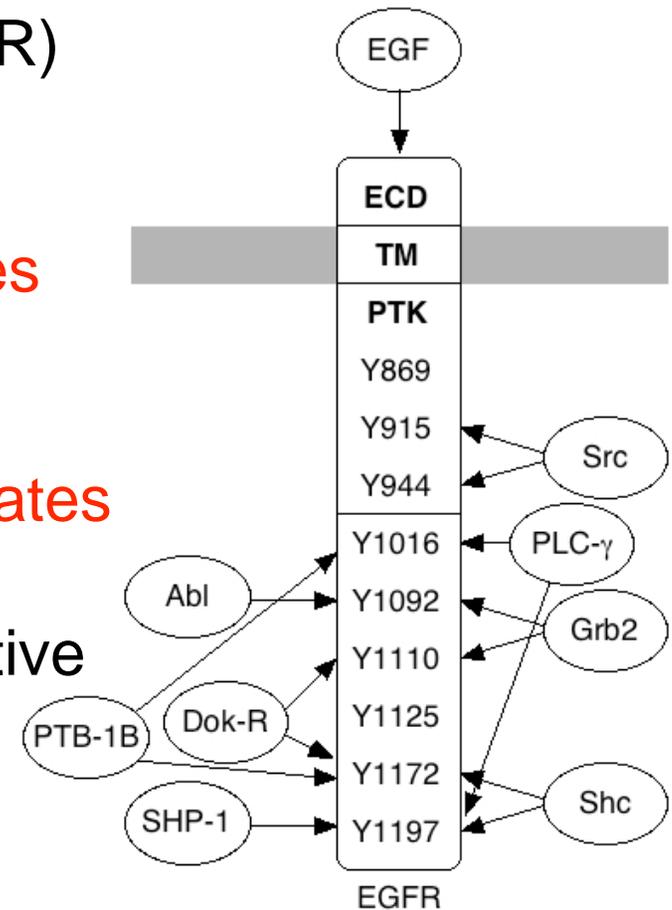
Multiplicity of sites and binding partners gives rise to combinatorial complexity

Epidermal growth factor receptor (EGFR)

9 sites $\Rightarrow 2^9=512$ phosphorylation states

Each site has ≥ 1 binding partner
 \Rightarrow more than $3^9=19,683$ total states

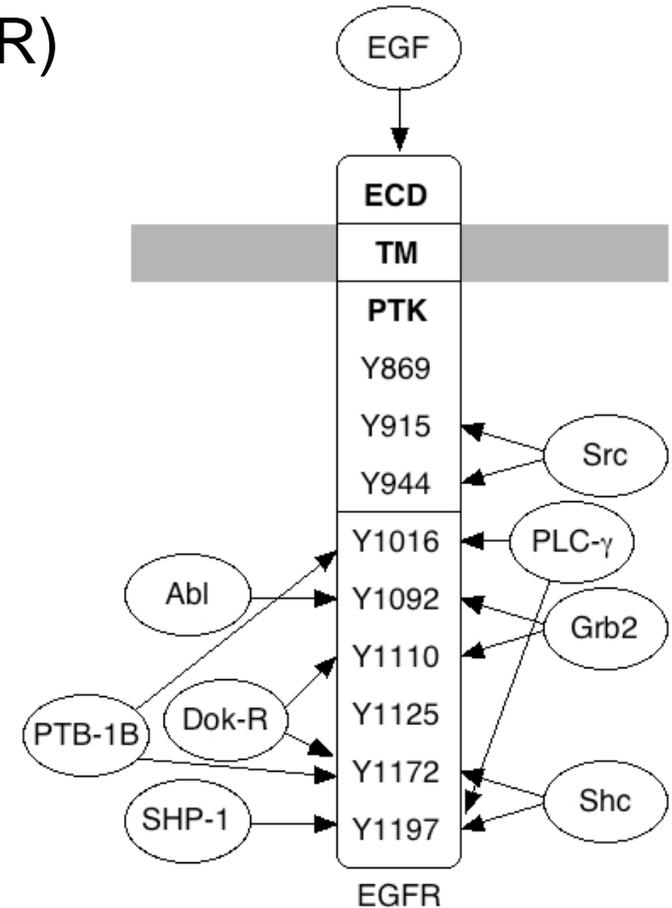
EGFR must form *dimers* to become active
 \Rightarrow more than 1.9×10^8 states



Multiplicity of sites and binding partners gives rise to combinatorial complexity

Epidermal growth factor receptor (EGFR)

...but the number of interactions is relatively small.



Summary

What functional role do protein domains and motifs play in signaling?

Combinatorial complexity

- *Modularity of protein structure*
- *Multivalent interactions*

BioNetGen language provides explicit representation of molecules and interactions

Molecules are *structured objects* (hierarchical graphs)



BNGL:

$A(b, Y1)$

$B(a)$

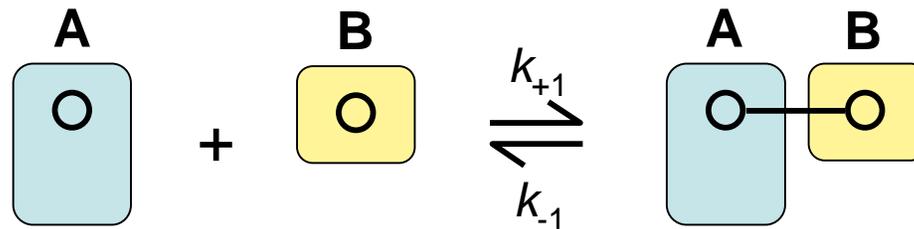
BioNetGen language provides explicit representation of molecules and interactions

Molecules are *structured objects* (hierarchical graphs)



BNGL: $A(b, Y1)$ $B(a)$

Rules define interactions (graph rewriting rules)

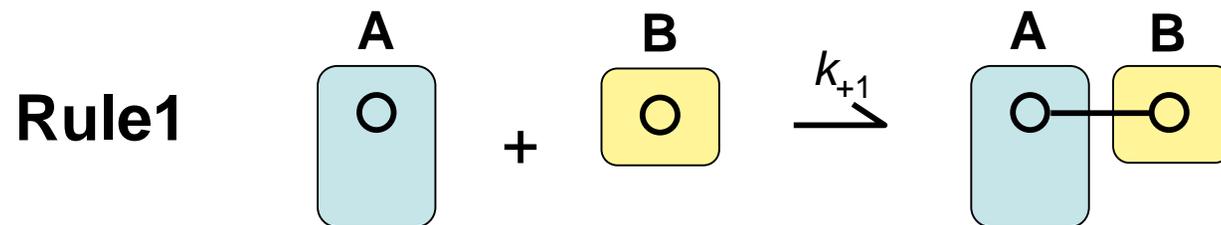


BNGL: $A(b) + B(a) \rightleftharpoons A(b!1) \cdot \underline{B(a!1)}$ $kp1, km1$

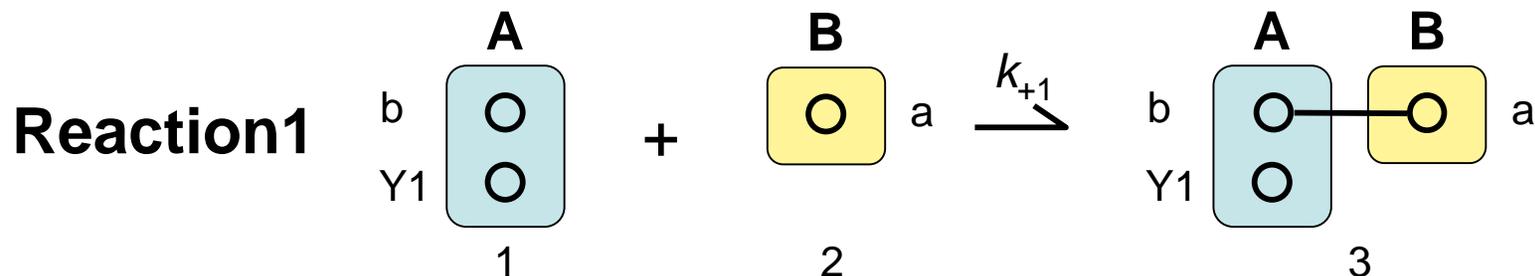
a bond between two components

Rules generate events

Example of reaction generation:

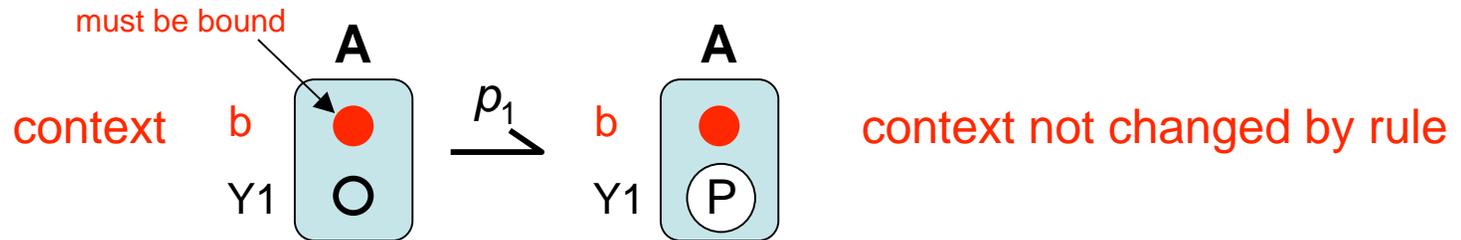


Rule1 applied to $\left\{ \begin{array}{cc} \text{A} & \text{B} \\ \text{b} & \text{a} \\ \text{Y1} & \\ \text{1} & \text{2} \end{array} \right\}$ generates



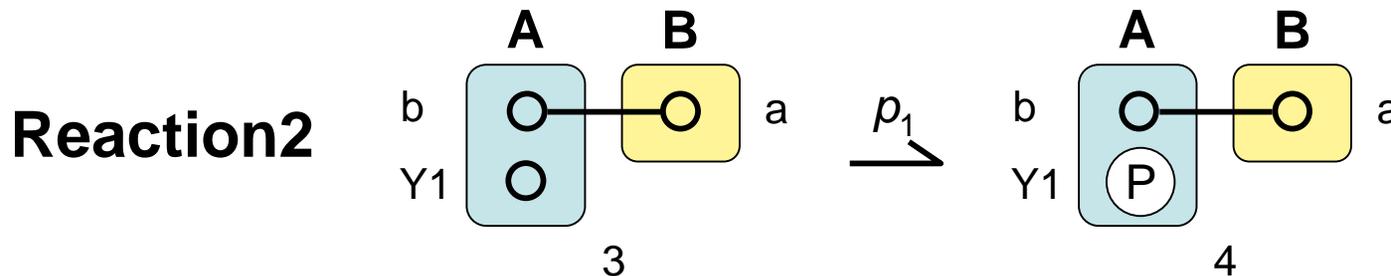
Rules may specify contextual requirements

Rule2



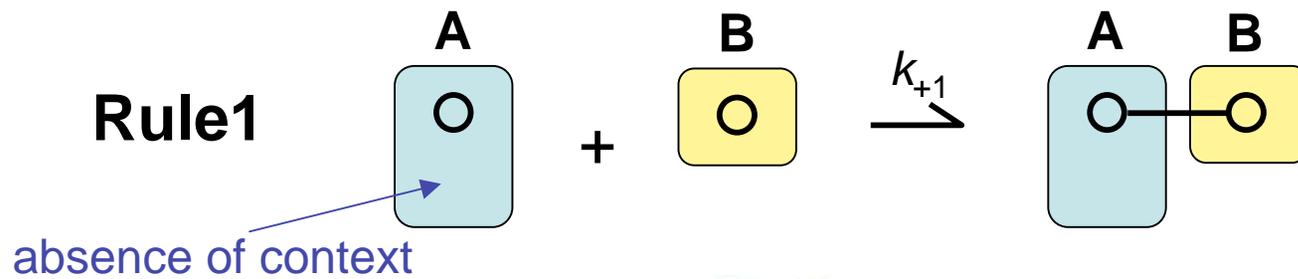
BNGL: $A(b!+, Y1 \sim U) \leftrightarrow A(b!+, Y1 \sim P) \quad p_1$

Rule2 applied to $\left\{ \begin{array}{c} \text{A} \quad \text{B} \\ \text{b} \quad \text{a} \\ \text{Y1} \quad \text{O} \\ \text{O} \quad \text{O} \\ \text{3} \end{array} \right\}$ generates

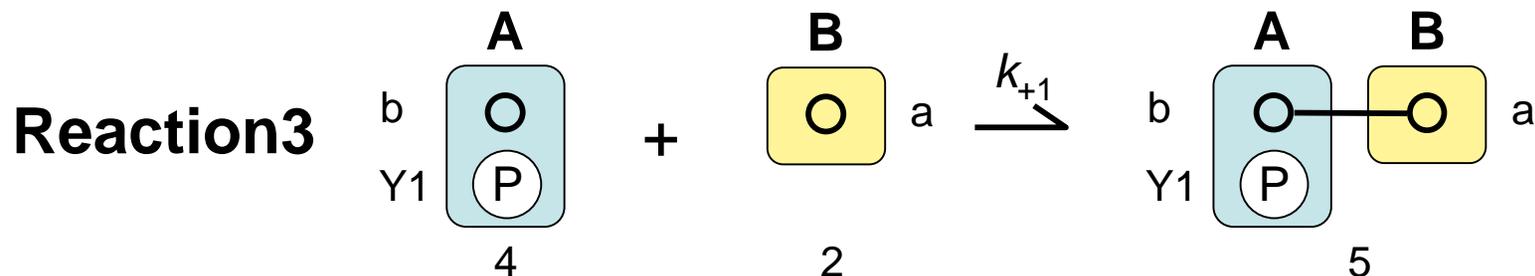


Rules may generate multiple events

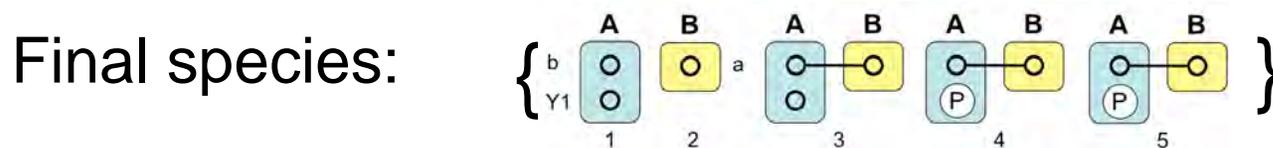
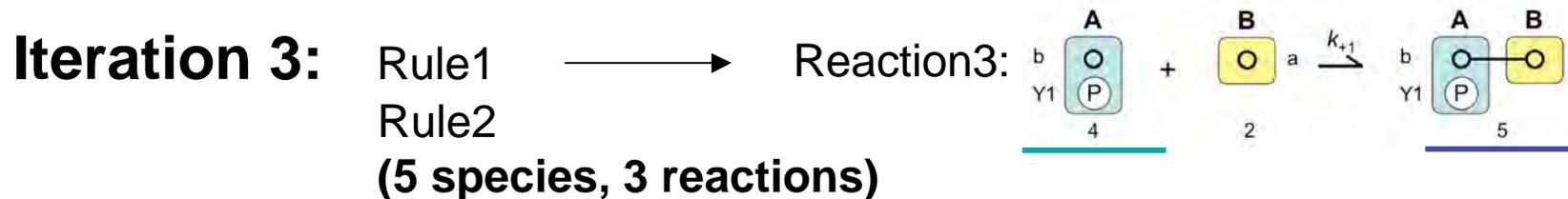
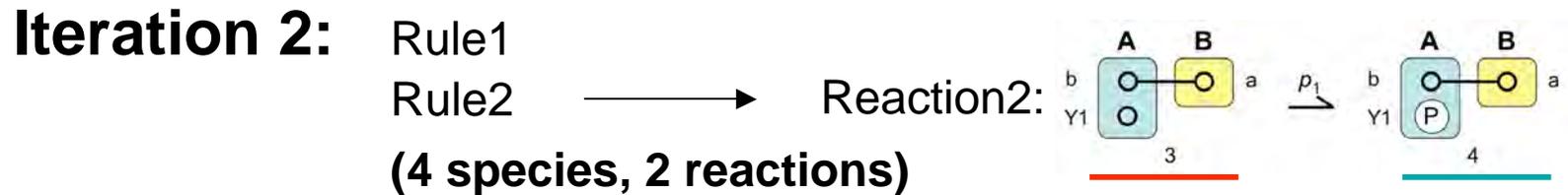
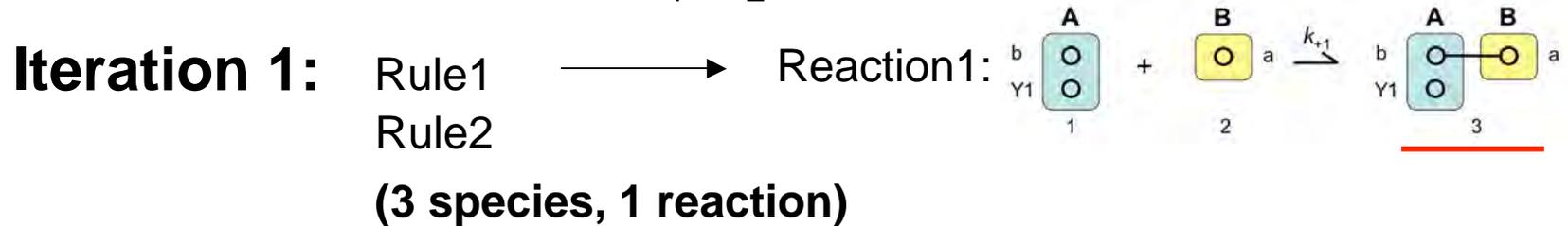
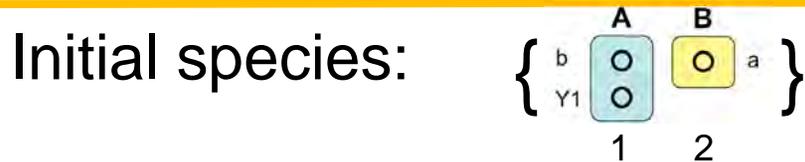
Second example of reaction generation:



Rule1 applied to $\left\{ \begin{matrix} \text{A} & \text{B} \\ \text{O} & \text{O} \\ \text{P} & \\ \text{4} & \text{2} \end{matrix} \right\}$ generates



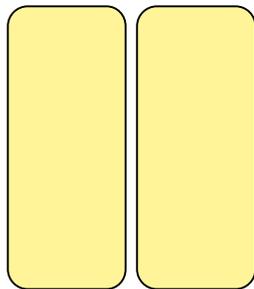
Iterative application of rules generates standard mass action reaction network



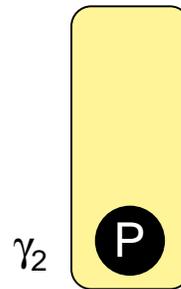
Observables use patterns to define model outputs

- Microscopic species generated by applying rules to molecules are difficult to observe directly.
- *Observables* define quantities that can be measured in experiments.

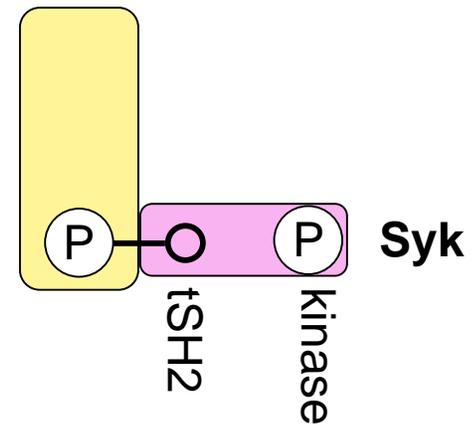
FcεRI



Receptor dimerization



γ_2 phosphorylation



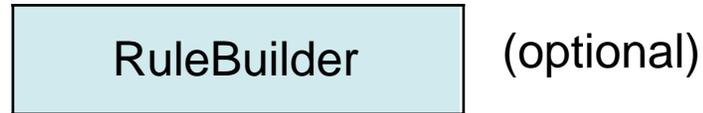
Syk activation

Elements of BNG Model

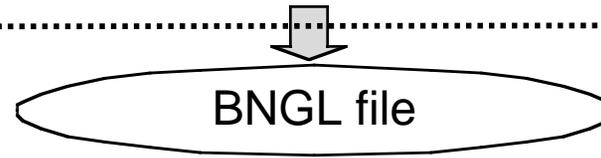
- **parameters**
 - defined anywhere
 - math expressions provide annotation
- **seed species**
 - Any molecule with non-zero initial concentration
- **reaction rules**
- **observables**
 - define model outputs
- **actions**
 - network generation
 - simulation
 - output
 - change parameters

BioNetGen2: Software for graphical rule-based modeling

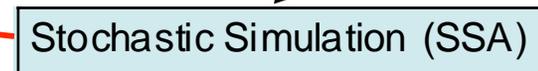
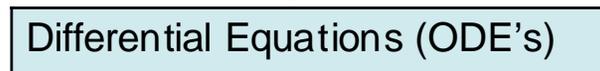
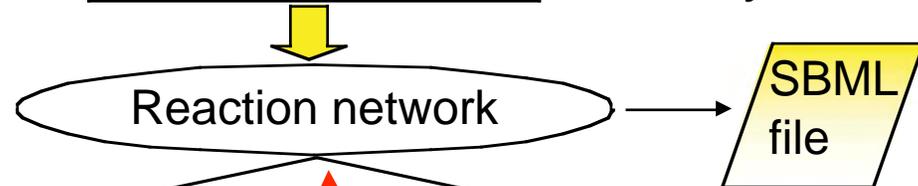
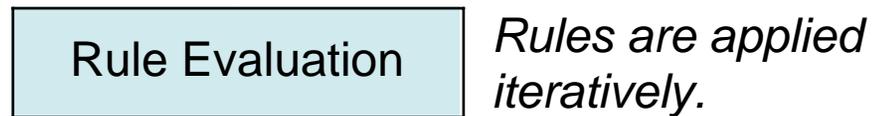
Graphical interface for composing rules



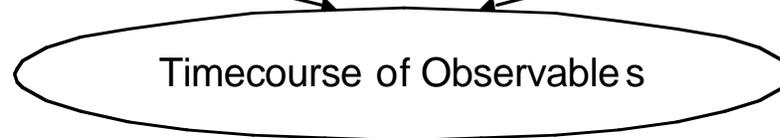
Text-based language



Simulation engine



'on-the-fly'



Advantages of BNGL

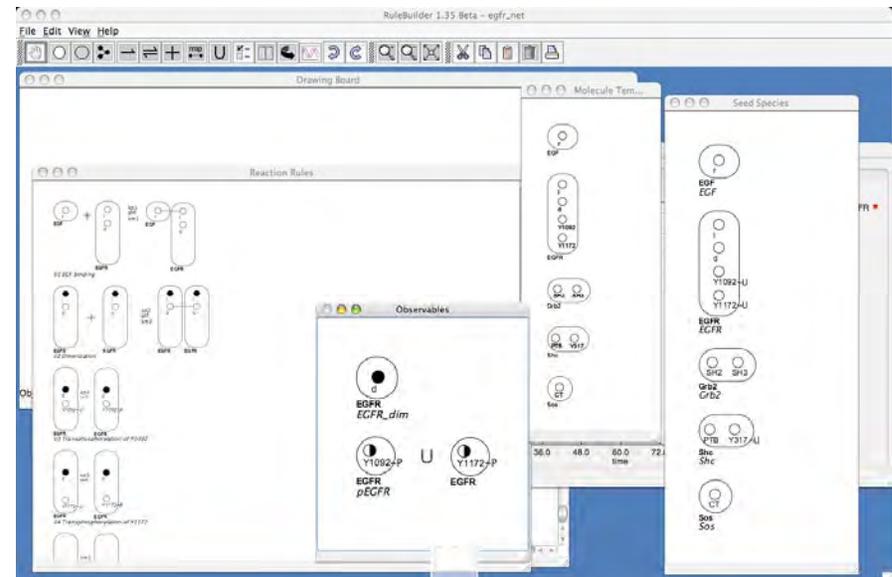
- Precise and flexible modeling language
- *Human readable*
 - Rules can be embedded in wikis, databases, applications, and papers (Ty Thomson)
- *Machine readable*
 - Forms basis for SBML L3 proposal (Blinov)
 - Interoperability (Vcell, Dynstoc, Kappa Factory, ...)
 - Molecule and rule definition could be automated using databases of protein-protein interactions as a source

Two interfaces to BNG

Terminal interface (text-based input)

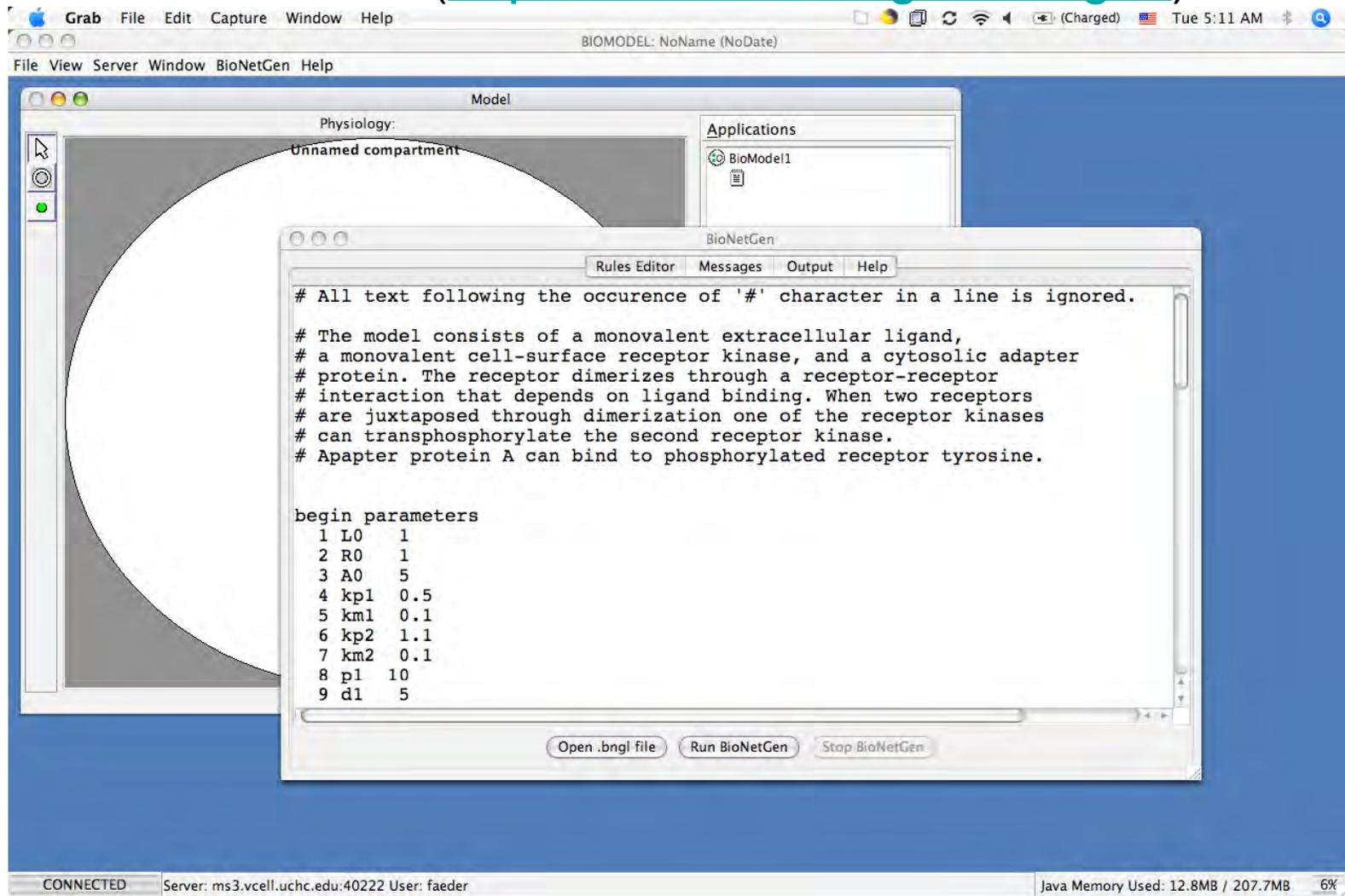
```
ntal:~/shared/Conferences/RTK-trainingcourse2006 faeder$ BNG2 AB.bngl
/Users/faeder/BioNetGen_2.0.40/Perl2/BNG2.pl
BioNetGen version 2.0.40
Reading from file AB.bngl
Read 1 parameters.
Read 2 species.
Read 1 reaction rule(s).
WARNING: Removing old network file AB.net.
Iteration 0: 2 species 0 rxns 0.00e+00 CPU s
Iteration 1: 3 species 1 rxns 0.00e+00 CPU s
Iteration 2: 3 species 1 rxns 0.00e+00 CPU s
Cumulative CPU time for each rule
Rule 1: 1 reactions 0.00e+00 CPU s 0.00e+00 CPU s/rxn
Total : 1 reactions 0.00e+00 CPU s 0.00e+00 CPU s/rxn
Wrote network to AB.net.
CPU TIME: generate_network 0.0 s.
Network simulation using ODEs
Running run_network on ntal.local
full command: "/Users/faeder/BioNetGen_2.0.40/bin/run_network_mac" -o "AB" -p cvoid -a 1e-08 -r 1e-08 -g "AB.net" "AB.net"
0.5 2
Read 1 parameters
Read 3 species
Read 1 reaction(s)
1 reaction(s) have nonzero rate
Read 0 group(s) from AB.net
Initialization took 0.00 CPU seconds
Propagating with cvoid using dense LU
      time  n_steps n_deriv_calls
0.50      308      355
1.00      352      404
Time course of concentrations written to file AB.cdat.
Propagation took 0.00 CPU seconds
Program times: 0.00 CPU s 0.00 clock s
Updating species concentrations from AB.cdat
CPU TIME: simulate_ode 0.0 s.
Finished processing file AB.bngl
CPU TIME: total 0.3 s.
ntal:~/shared/Conferences/RTK-trainingcourse2006 faeder$
```

RuleBuilder GUI



A Third Way - Virtual Cell Interface

BioNetGen@Vcell (<http://www.vcell.org/bionetgen>)



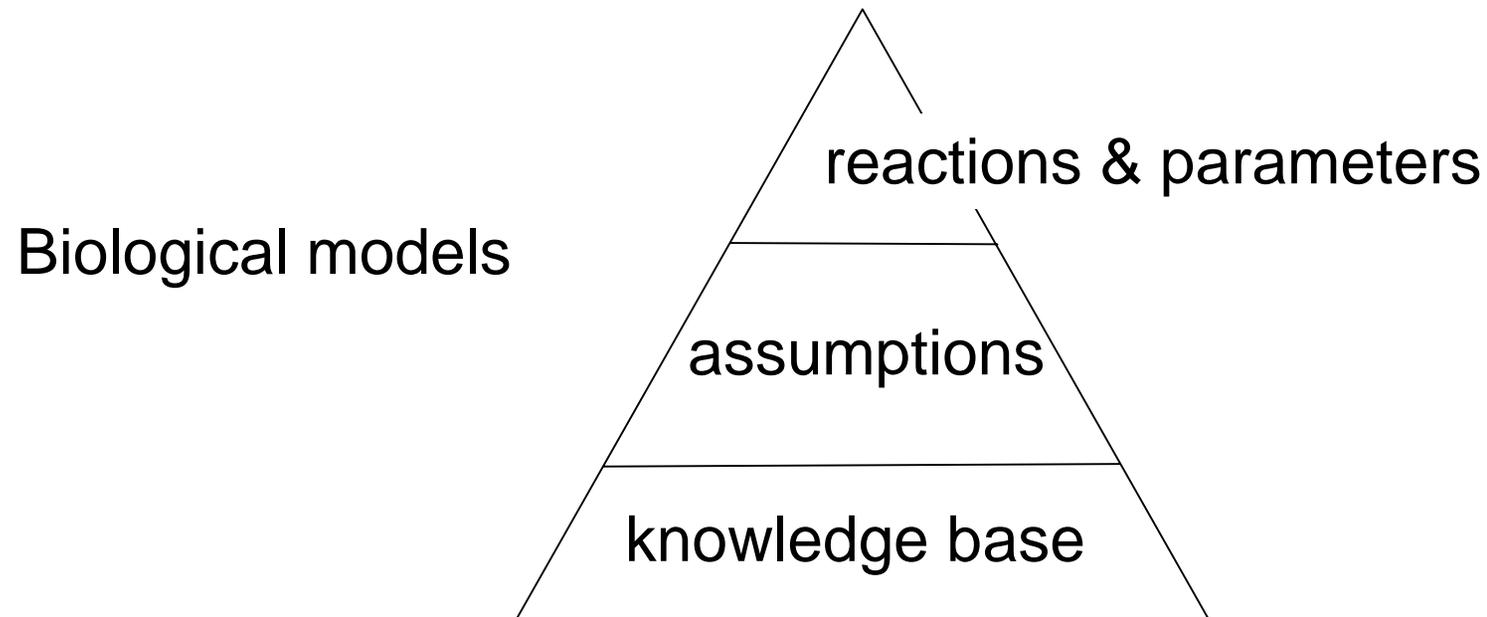
The screenshot displays the BioNetGen@Vcell interface. The main window, titled "Model", shows a "Physiology:" section with an "Unnamed compartment" and a large white circular area. A "BioNetGen" window is open in the foreground, showing a "Rules Editor" with the following text:

```
# All text following the occurrence of '#' character in a line is ignored.  
  
# The model consists of a monovalent extracellular ligand,  
# a monovalent cell-surface receptor kinase, and a cytosolic adapter  
# protein. The receptor dimerizes through a receptor-receptor  
# interaction that depends on ligand binding. When two receptors  
# are juxtaposed through dimerization one of the receptor kinases  
# can transphosphorylate the second receptor kinase.  
# Adapter protein A can bind to phosphorylated receptor tyrosine.  
  
begin parameters  
1 L0 1  
2 R0 1  
3 A0 5  
4 kp1 0.5  
5 km1 0.1  
6 kp2 1.1  
7 km2 0.1  
8 p1 10  
9 d1 5
```

At the bottom of the BioNetGen window are buttons for "Open .bnl file", "Run BioNetGen", and "Stop BioNetGen". The status bar at the bottom of the interface shows "CONNECTED", "Server: ms3.vcell.uhc.edu:40222 User: faeder", and "Java Memory Used: 12.8MB / 207.7MB 6%".

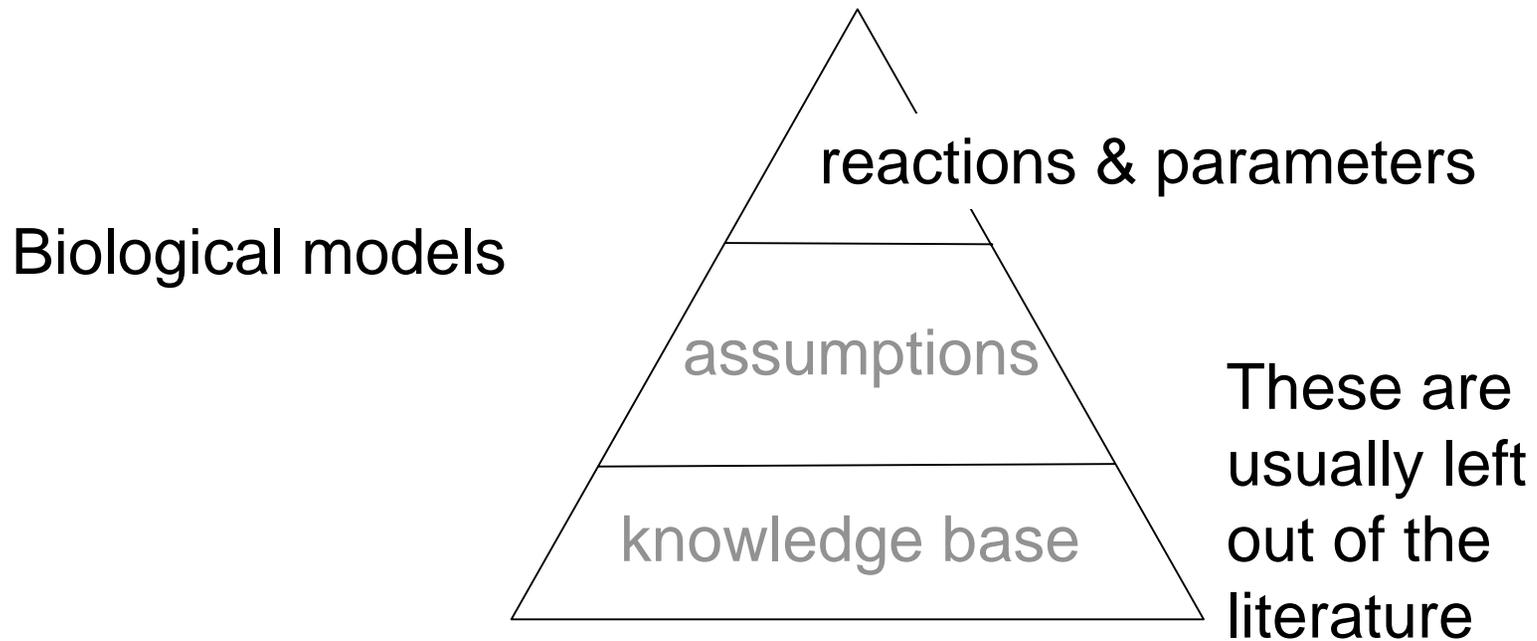
The AlphaWiki Yeast Pheromone Response Model

Ty Thomson & Drew Endy (MIT)



The AlphaWiki Yeast Pheromone Response Model

Ty Thomson & Drew Endy (MIT)



The AlphaWiki Yeast Pheromone Response Model

Ty Thomson & Drew Endy (MIT)

Structured
wiki may offer
a solution

The screenshot shows a web browser window with the URL http://yeastpheromonemodel.org/wiki/Pheromone/Receptor/G_protein_interactions. The page title is "Pheromone/Receptor/G protein interactions". On the left side, there is a navigation menu with links for "Main Page", "Recent changes", and "Help". Below that is a search box with "Go" and "Search" buttons. A "toolbox" section contains links for "What links here", "Related changes", "Upload file", "Special pages", "Printable version", and "Permanent link". The main content area features a "Contents" table of contents with sections: "1 Receptor interactions with pheromone" (sub-sections: 1.1 Measured Binding Affinities, 1.2 Effect of Gα on receptor/pheromone binding affinity, 1.3 Reaction Definition), "2 Receptor interactions with G protein" (sub-section: 2.1 Reaction Definition), and "3 Gα (Gpa1) interactions with Gβγ (Ste4:Ste18)" (sub-section: 3.1 Reaction Definition). The "Receptor interactions with pheromone" section is expanded, showing a list of references: "■ Coexpression of a *Ste2* mutant that is defective in pheromone binding (S184R) and a *Ste2* mutant that is defective in G protein binding (specific alanine replacement in any of the 3 intracellular loops along with C terminal truncation at residue 303) does not restore sensitivity to pheromone in a *ste2Δ* strain. This was judged by halo formation and *Fus1-lacZ* expression. Chinault et al. 2004 PMID 14764600", "■ This suggests that receptors in a dimer/oligomer are independently activated by binding to their own pheromone peptide, rather than signaling in trans by allowing binding of pheromone to one *Ste2* in a dimer to activate the other *Ste2* in the dimer.", and "■ These mutants were shown to form hetero-oligomers by FRET." Below this, the "Measured Binding Affinities" section is expanded, showing a list of references: "■ Kd = 6.4 nM when *Ste2* is expressed off a multicopy plasmid (measured at 0°C). The strains used were *bart1*-to prevent pheromone degradation. Bajaj et al. 2004 PMID 15491163", "■ Using a fluorescent pheromone analog [K⁷(NBD),Nle¹²] α-factor, Kd = 3.6 nM when *Ste2* is expressed from a CEN plasmid, and Kd = 7.4 nM when *Ste2* is expressed from a multicopy plasmid. For *Ste2* expressed off the multicopy plasmid, the on and off rates were also measured: kon = 1.6 * 10⁶ M⁻¹s⁻¹, koff = 1.1 * 10⁻³ s⁻¹. These values were all measured at 0°C.", "■ The fluorescent pheromone analog's binding kinetics fits better to a double exponential than to a single exponential.", "■ Kd = 6 nM. Experiment was done using ³⁵S-labeled pheromone at 22°C. TAME was used to prevent pheromone degradation, and cells were treated with NaN₃ and KF to prevent growth and other energy-dependent processes. Jenness et al. 1986 PMID 3023832", "■ koff = 9 * 10⁻⁴ s⁻¹. Experiment was done using ³⁵S-labeled pheromone at 22°C. TAME was used to prevent pheromone degradation, and cells were treated with NaN₃ and KF to prevent growth and other energy-dependent processes. Kd was also measured, but later discounted by the same group. Jenness et al. 1983 PMID 6360378", "■ Kd = 7 nM +/- 1 nM (measured in triplicate). Experiments were done with ³H-labeled pheromone at 22°C. TAME was used to prevent pheromone degradation, and cells were treated with NaN₃ and KF to prevent growth and other energy-dependent processes. David et al. 1997 PMID 9142592", "■ Kd = 4.2 nM. Experiments were done with ³⁵S-labeled pheromone, and the cells were treated with NaN₃ and KF. The strains used contained a non-functional *bart1*-1 allele to prevent pheromone degradation. The experiment was performed at room temperature. Dossil et al. 2000 PMID 10366888", and "■ Kd = 4.5 nM. Experiments were done with ³⁵S-labeled pheromone, and the cells were treated with NaN₃ and KF. The strains used contained a non-functional *bart1*-1 allele to".

Done

The AlphaWiki Yeast Pheromone Response Model

Ty Thomson & Drew Endy (MIT)

Receptor interactions with G protein [edit]

- [Gpa1](#) contains a [Ste2](#) binding domain. Kallal et al. 1997 PMID 9111362
- The accepted model for [G protein](#) coupled receptors is that when ligand-bound, the [receptor](#) acts as a guanine nucleotide exchange factor (GEF) for the [G protein](#). Nucleotide exchange results in the binding of GTP to the [Gα](#) subunit ([Gpa1](#)), which activates the [G protein](#) leading to [Gα](#) ([Gpa1](#)) dissociation from both [Gβγ](#) ([Ste4:Ste18](#)) and the receptor ([Ste2](#)). Preininger and Hamm 2004 PMID 14762218
- [G protein](#) coupling to [Ste2](#) *in vivo* requires the presence of functional [Ste4:Ste18](#) and functional [Gpa1](#). Blumer and Thorer 1990 PMID 2161538
- [Ste2](#) mutated in the C-terminal cytosolic tail (N388S) exhibits reduced interaction with [Ste4](#) and [Gpa1](#), as determined by 2-hybrid assay. Duran-Avelar et al. 2001 PMID 11287148
- Upon [pheromone](#) treatment, less [Gpa1](#) is associated with [Ste2](#) than prior to treatment (co-IP). Wu et al. 2004 PMID 15197187
- Coexpression of a [Ste2](#) mutant (alanine substitutions in the 1st intracellular loop) that is thought to be defective in [Gpa1](#) binding/activation with another [Ste2](#) mutant (alanine substitutions in the 3rd intracellular loop) that is thought to be defective in [Gpa1](#) binding/activation partially restores sensitivity to [pheromone](#). Chinault et al. 2004 PMID 14764600
 - The authors conclude from this that [Ste2](#) monomers in a dimers cooperate to activate [Gpa1](#).

Reaction Definition [edit]

We know that [Gpa1](#) contains a [Ste2](#) binding domain, so presumably the coupling of the [G protein](#) to the receptor is through [Gpa1](#). We also know that [Gpa1](#) couples inefficiently to [Ste2](#) in the absence of [Ste4:Ste18](#). This leads to a model where [Gpa1](#) binds [Ste2](#) weakly, and [Ste4:Ste18](#) greatly increases this binding efficiency. In other words, [Gpa1](#) (GDP), which is usually present in the heterotrimeric [Gpa1:Ste4:Ste18](#) complex, binds to [Ste2](#) with much higher affinity than [Gpa1](#) (GTP), which is usually present free of binding to [Ste4:Ste18](#). A consequence of this relative affinity is that a single molecule or dimer of [Ste2](#) could act enzymatically to catalyze the nucleotide exchange on many [Gpa1](#) molecules.

Assumptions:

- Above we've assumed that [α-factor](#) binding is not affected by the [G protein](#), which is equivalent to assuming that [G protein](#) binding to [Ste2](#) is not affected by [pheromone](#).
- [Ste2](#)'s phosphorylation state has no effect on [G protein](#) coupling and [Yck](#) binding does not affect [G protein](#) coupling.
- [Gpa1](#)'s nucleotide state only indirectly affects [G protein/receptor](#) coupling by affecting [Gpa1](#)'s affinity for [Ste4:Ste18](#).
- [Sst2](#) binding to [Ste2](#) does not affect [G protein](#) binding to [Ste2](#) (see [RGS\(Sst2\)/Gα\(Gpa1\)/Receptor\(Ste2\)](#) interactions)
- The difference in affinity between [Gpa1/Ste2](#) and [Gpa1:Ste4:Ste18/Ste2](#) arises solely as differences in off-rates.

$$\text{Ste2}(\text{Gpa1_site}) + \text{Gpa1}(\text{Ste2_site}, \text{ste4_site}) \leftrightarrow \text{Ste2}(\text{Gpa1_site}!1).\text{Gpa1}(\text{Ste2_site}!1, \text{ste4_site})$$

- Forward rate constant [kon_Ste2_Gpa1](#)
- Reverse rate constant [koff_Ste2_Gpa1](#)

$$\text{Ste2}(\text{Gpa1_site}) + \text{Gpa1}(\text{Ste2_site}, \text{ste4_site}!+) \leftrightarrow \text{Ste2}(\text{Gpa1_site}!1).\text{Gpa1}(\text{Ste2_site}!1, \text{ste4_site}!+)$$

- Forward rate constant [kon_Ste2_Gpa1Ste4Ste18](#)
- Reverse rate constant [koff_Ste2_Gpa1Ste4Ste18](#)

There are [specific constraints](#) on these rate constants.

BNG rules are used for precise reaction definitions

The AlphaWiki Yeast Pheromone Response Model

Ty Thomson & Drew Endy (MIT)

Pheromone/Receptor/G protein interactions – Yeast Pheromone Model

http://yeastpheromonemodel.org/wiki/Pheromone/Receptor/G_protein_interactions

Receptor interactions with G protein [edit]

- [Gpa1](#) contains a [Ste2](#) binding domain. Kallal et al. 1997 PMID 9111362 [↗](#)
- The accepted model for [G protein](#) coupled receptors is that when ligand-bound, the [receptor](#) acts as a guanine nucleotide exchange factor (GEF) for the [G protein](#). Nucleotide exchange results in the binding of GTP to the [Gα](#) subunit ([Gpa1](#)), which activates the [G protein](#) leading to [Gα](#) ([Gpa1](#)) dissociation from both [Gβγ](#) ([Ste4:Ste18](#)) and the receptor ([Ste2](#)). Preinger and Hamm 2004 PMID 14762218 [↗](#)
- [G protein](#) coupling to [Ste2](#) *in vivo* requires the presence of functional [Ste4:Ste18](#) and functional [Gpa1](#). Blumer and Thorer 1990 PMID 2161538 [↗](#)
- [Ste2](#) mutated in the C-terminal cytosolic tail (N388S) exhibits reduced interaction with [Ste4](#) and [Gpa1](#), as determined by 2-hybrid assay. Duran-Avelar et al. 2001 PMID 11287148 [↗](#)
- Upon [pheromone](#) treatment, less [Gpa1](#) is associated with [Ste2](#) than prior to treatment (co-IP). Wu et al. 2004 PMID 15197187 [↗](#)
- Coexpression of a [Ste2](#) mutant (alanine substitutions in the 1st intracellular loop) that is thought to be defective in [Gpa1](#) binding/activation with another [Ste2](#) mutant (alanine substitutions in the 3rd intracellular loop) that is thought to be defective in [Gpa1](#) binding/activation partially restores sensitivity to [pheromone](#). Chinault et al. 2004 PMID 14764600 [↗](#)
 - The authors conclude from this that [Ste2](#) monomers in a dimers cooperate to activate [Gpa1](#).

Reaction Definition [edit]

We know that [Gpa1](#) contains a [Ste2](#) binding domain, so presumably the coupling of the [G protein](#) to the receptor is through [Gpa1](#). We also know that [Gpa1](#) couples inefficiently to [Ste2](#) in the absence of [Ste4:Ste18](#). This leads to a model where [Gpa1](#) binds [Ste2](#) weakly, and [Ste4:Ste18](#) greatly increases this binding efficiency. In other words, [Gpa1](#)(GDP), which is usually present in the heterotrimeric [Gpa1:Ste4:Ste18](#) complex, binds to [Ste2](#) with much higher affinity than [Gpa1](#) (GTP), which is usually present free of binding to [Ste4:Ste18](#). A consequence of this relative affinity is that a single molecule or dimer of [Ste2](#) could act enzymatically to catalyze the nucleotide exchange on many [Gpa1](#) molecules.

Assumptions:

- Above we've assumed that [α-factor](#) binding is not affected by the [G protein](#), which is equivalent to assuming that [G protein](#) binding to [Ste2](#) is not affected by [pheromone](#).
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- Reverse rate constant [koff_Ste2_Gpa1Ste4Ste18](#)

There are [specific constraints](#) on these rate constants.

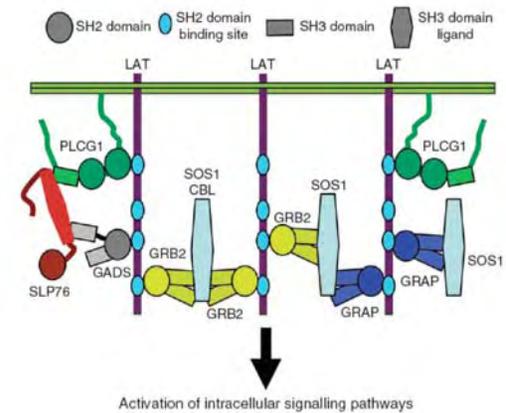
Model is automatically generated from wiki

Systems Modeled

- IgE Receptor (Fc ϵ RI)
 - Faeder et al. *J. Immunol.* (2003)
 - Goldstein et al. *Nat. Rev. Immunol.* (2004)
- Growth Factor Receptors
 - Blinov et al. *Biosyst.* (2006) [EGFR]
 - Barua et al. *Biophys. J.* (2006) [Shp2]
- TLR4, TCR, IFN γ , TNF- α , TGF- β , ...
- Carbon Fate Maps
 - Mu et al., submitted.

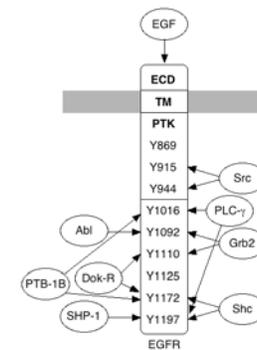
Key insights

- RBM's are straightforward to construct and do not require more parameters
 - New predictions
- Important role of multivalent interactions
 - Complex formation can produce ligand specificity (kinetic proofreading)
 - Intuition often fails
 - Oligomerization may be a common feature of biological signaling
- Concurrency in biological information processing
 - Scaffolds can activate multiple pathways independently
 - Strong potential for interaction among pathways (largely unexplored)

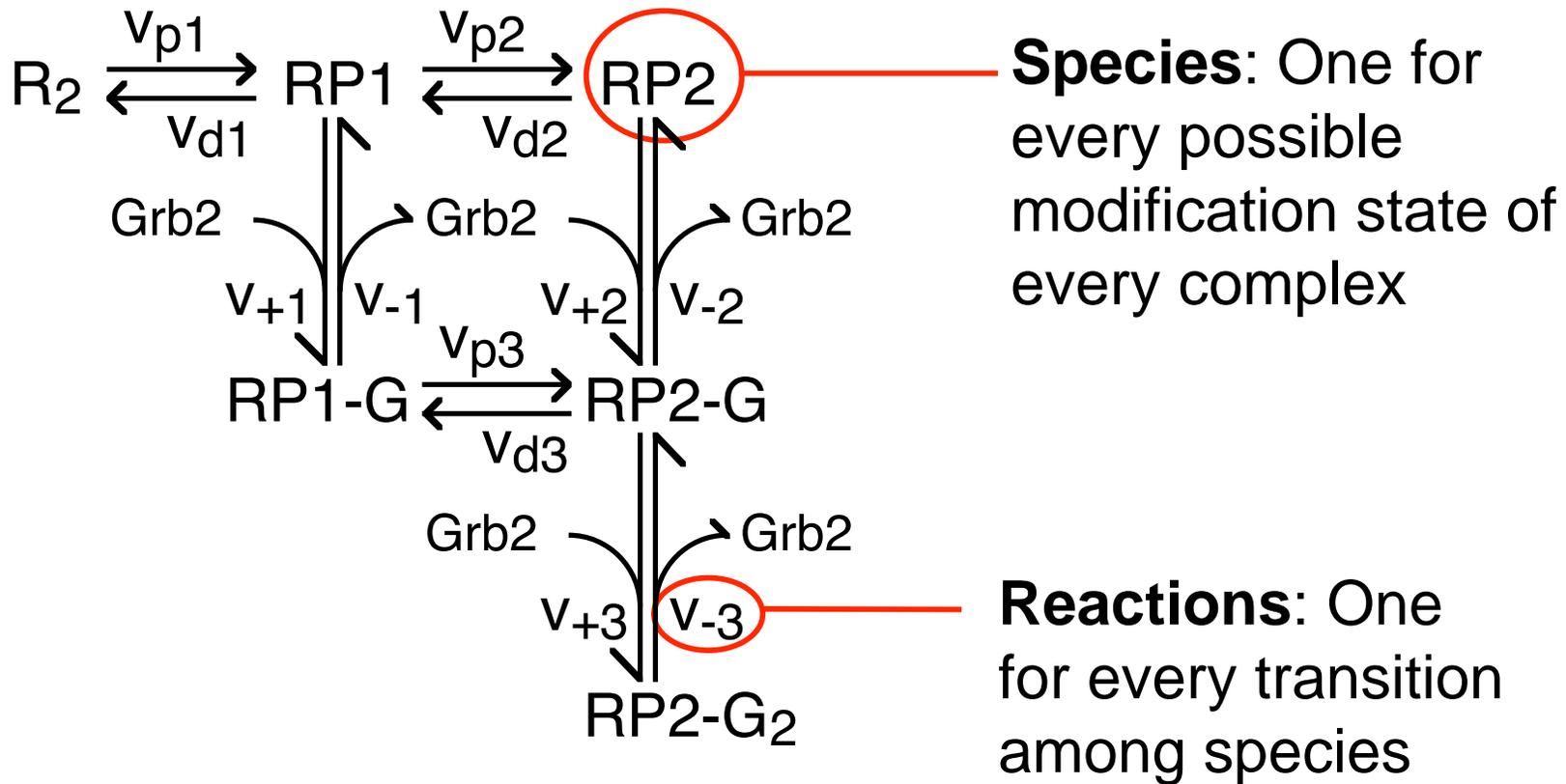


Activation of intracellular signalling pathways

Ambarish Nag



A standard reaction scheme

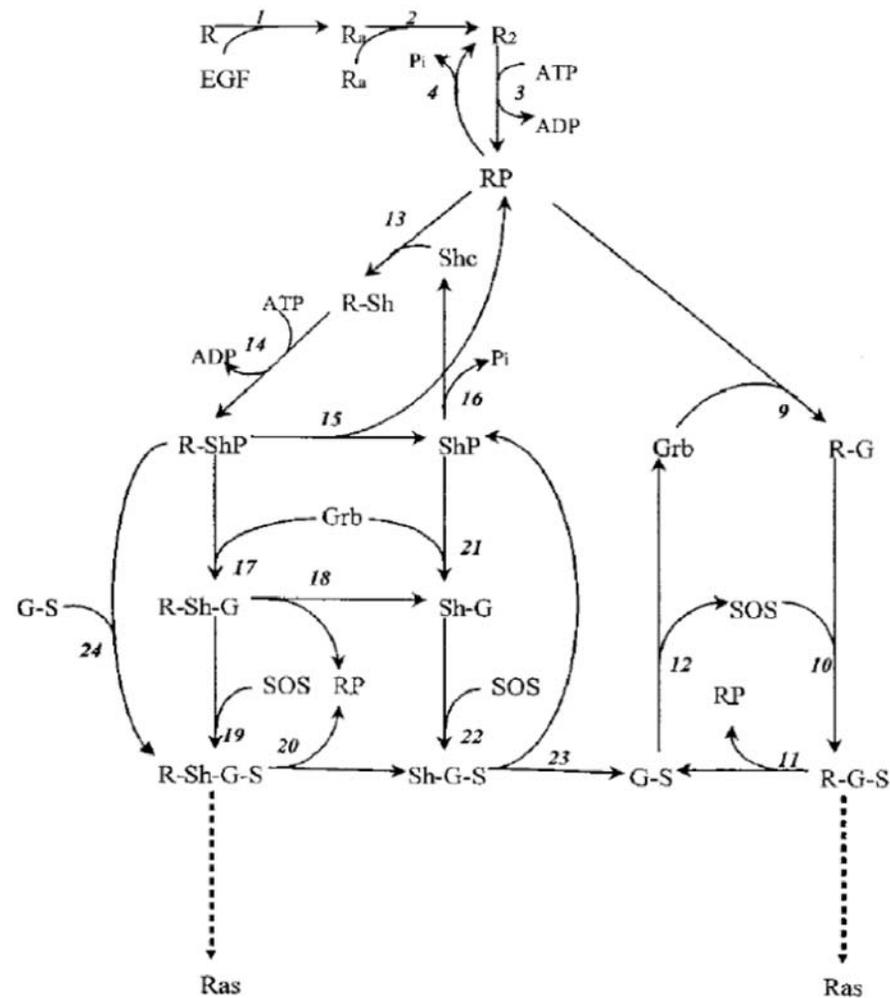


Mass action kinetics gives rise to a set of ODEs, one for each species

A conventional model for EGFR signaling

The Kholodenko model*

Avoids combinatorial complexity by assuming that certain reaction events must occur in a particular order

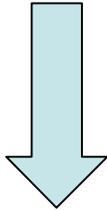


J. Biol. Chem.* **274, 30169 (1999)

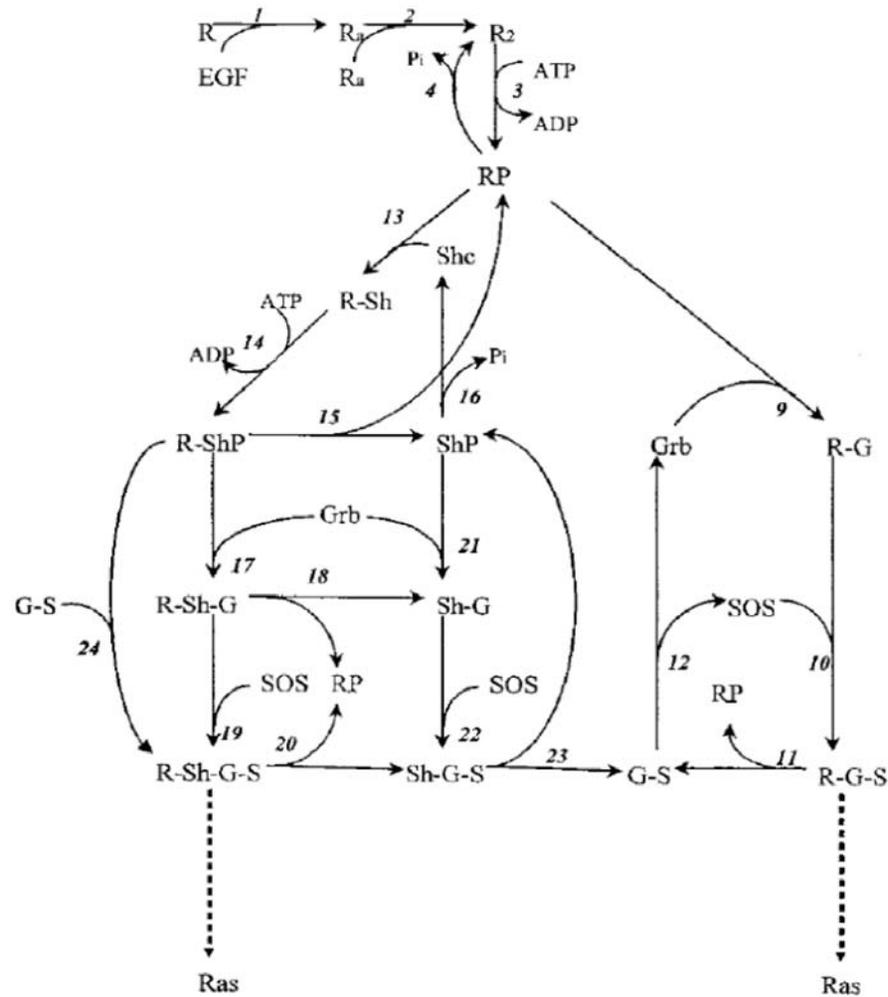
A conventional model for EGFR signaling

The Kholodenko model*

5 components

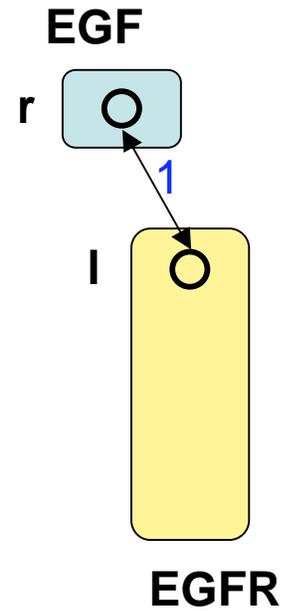


18 species
34 reactions



Dissecting the reaction scheme

1. EGF binding to EGFR



Dissecting the reaction scheme

1. EGF binding to EGFR

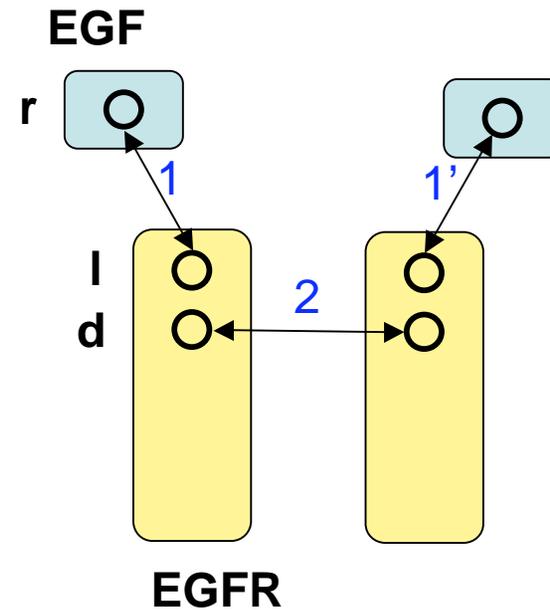


Dissecting the reaction scheme

1. EGF binding to EGFR

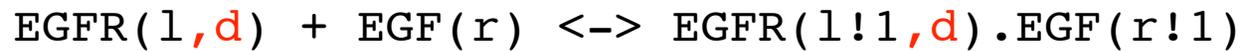


2. EGFR dimerization

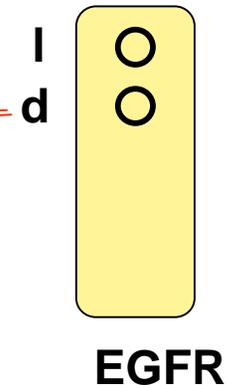


Dissecting the reaction scheme

1. EGF binding to EGFR



additional context because
representation is flat



2. EGFR dimerization

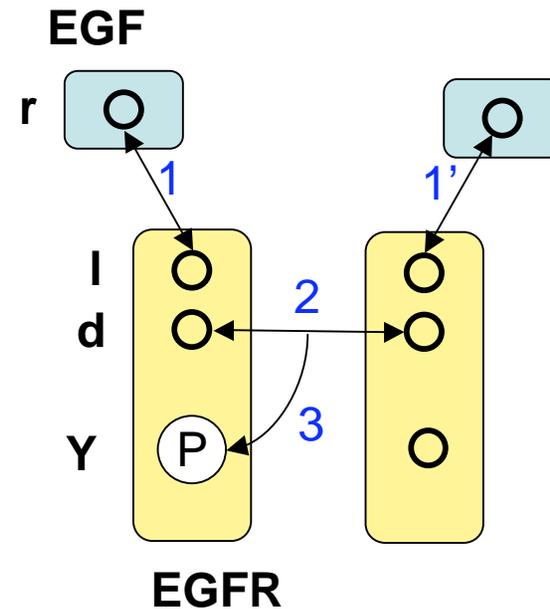


Dissecting the reaction scheme

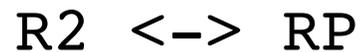
1. EGF binding to EGFR



2. EGFR dimerization

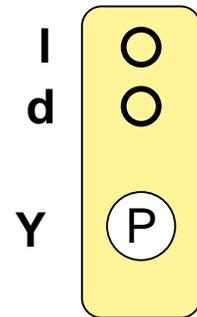
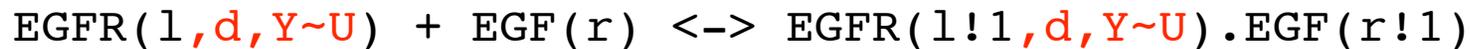


3. EGFR autophosphorylation



Dissecting the reaction scheme

1. EGF binding to EGFR

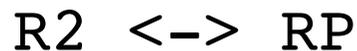


EGFR

2. EGFR dimerization



3. EGFR autophosphorylation

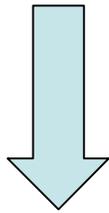


Assumptions accumulate!

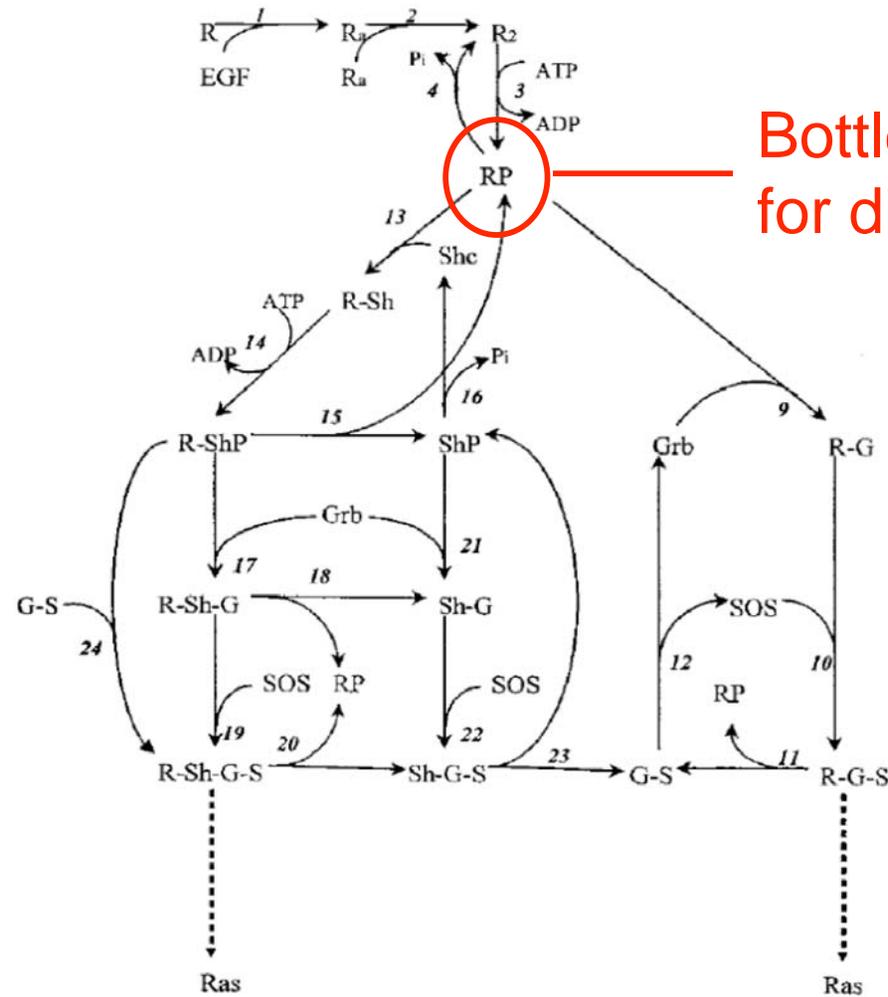
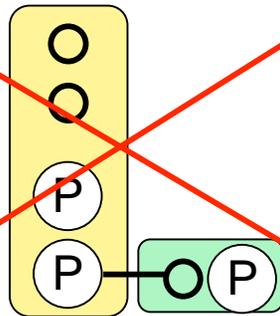


Effect of assuming receptor activation is sequential

1. Phosphorylation inhibits dimer breakup



No modified monomers



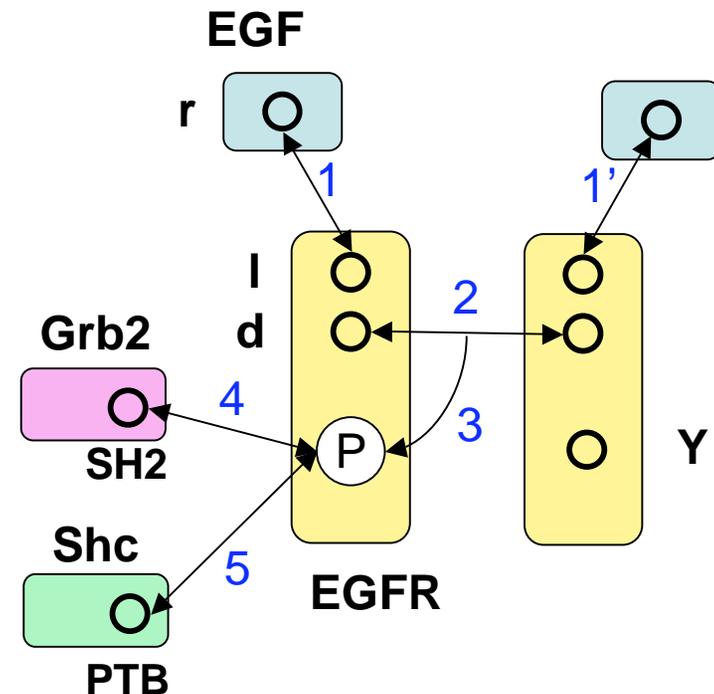
Bottleneck for dimers

Adaptor protein binding

4. Grb2 binding to pEGFR



5. Shc binding to pEGFR



Binding is assumed to be competitive

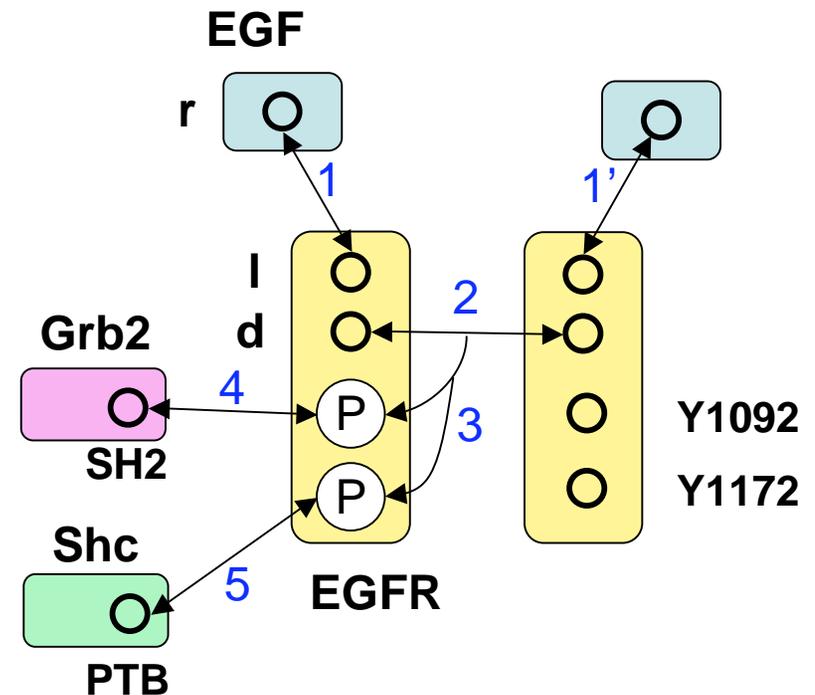
- either 4 or 5 may occur but not both
- only 1 adaptor per EGFR dimer

Splitting the adaptor binding site

4. Grb2 binding to pEGFR



5. Shc binding to pEGFR

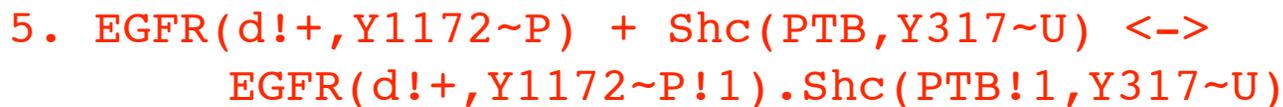
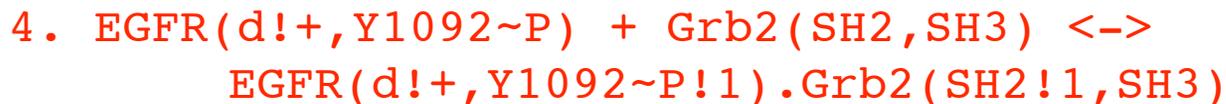
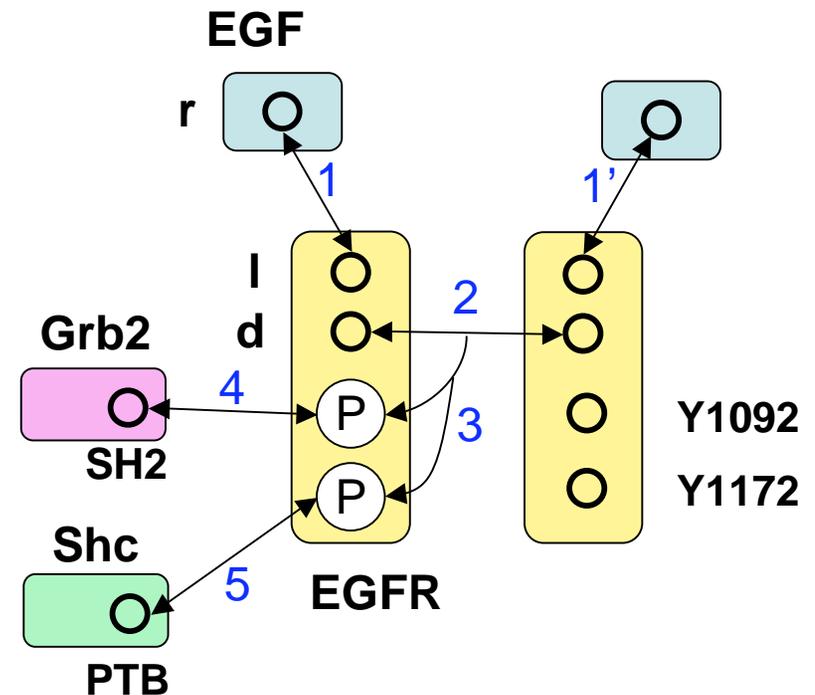


Splitting the adaptor binding site

4. Grb2 binding to pEGFR

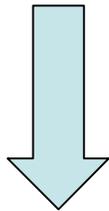


5. Shc binding to pEGFR

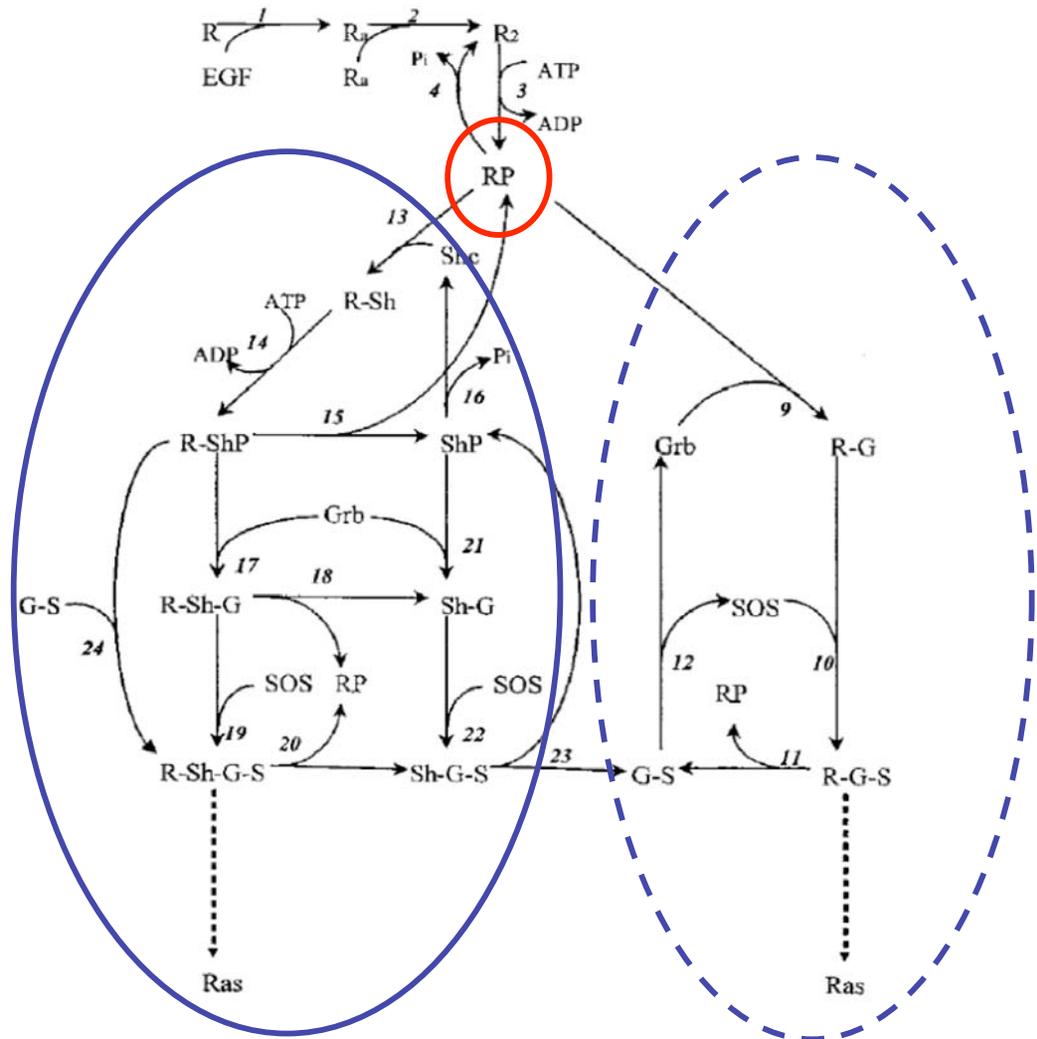
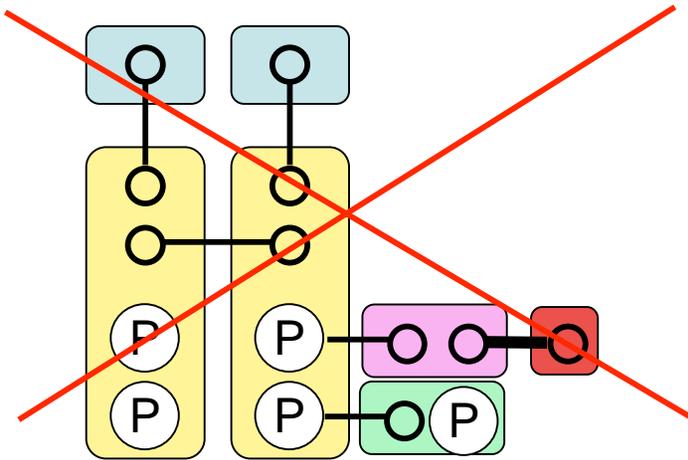


Effect of assuming adaptor binding is competitive

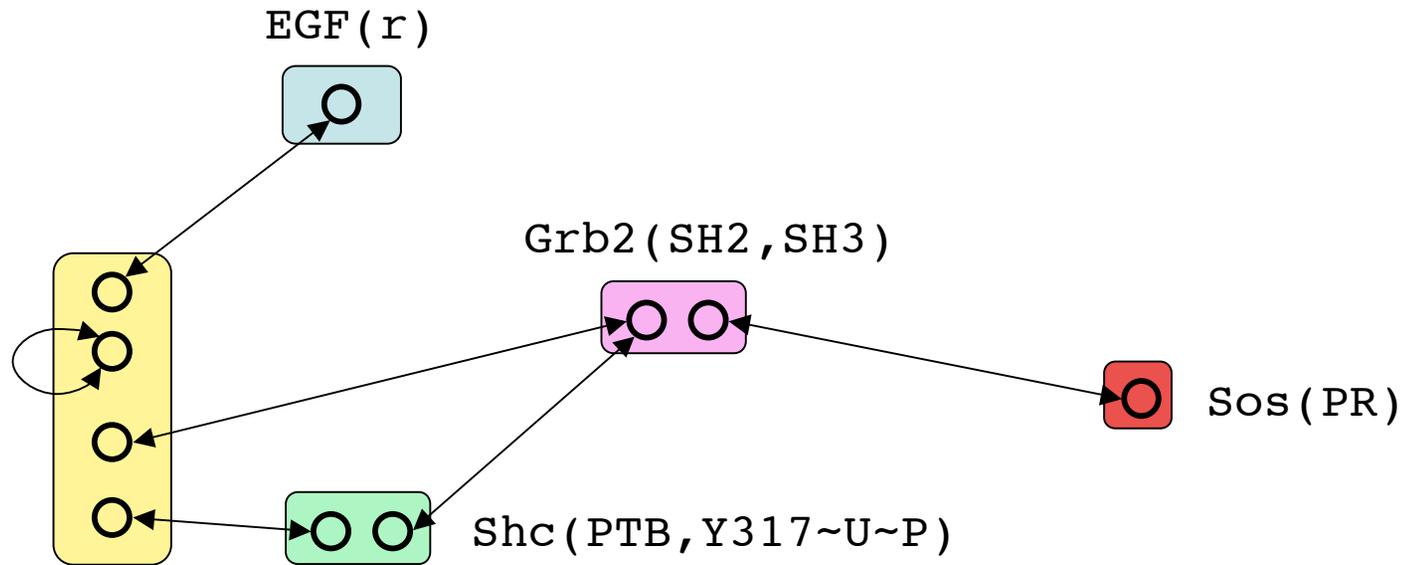
2. Adaptor binding is competitive



No dimers with more than one site modified



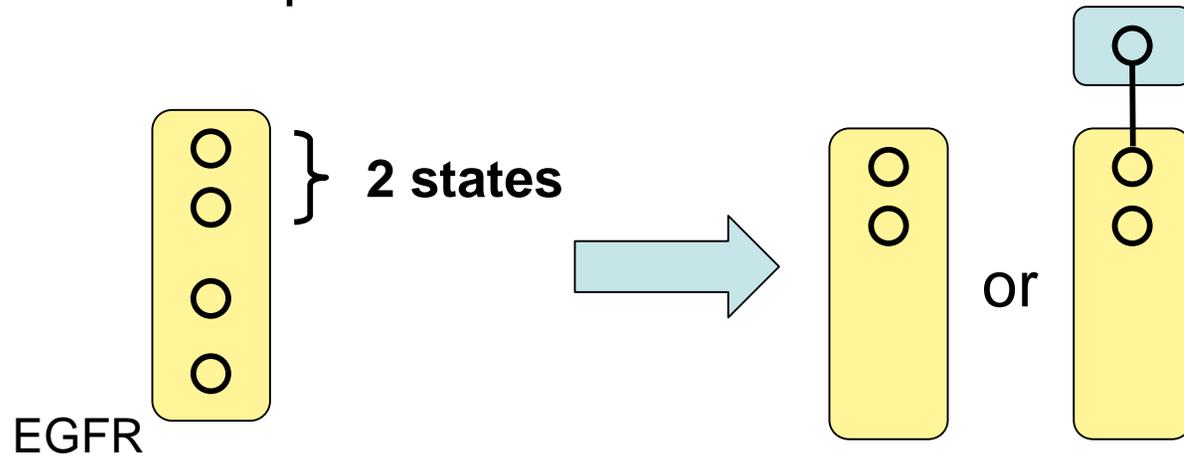
Molecules, components, and Interactions of the Kholodenko Model



EGFR (l, d, Y1092~U~P, Y1172~U~P)

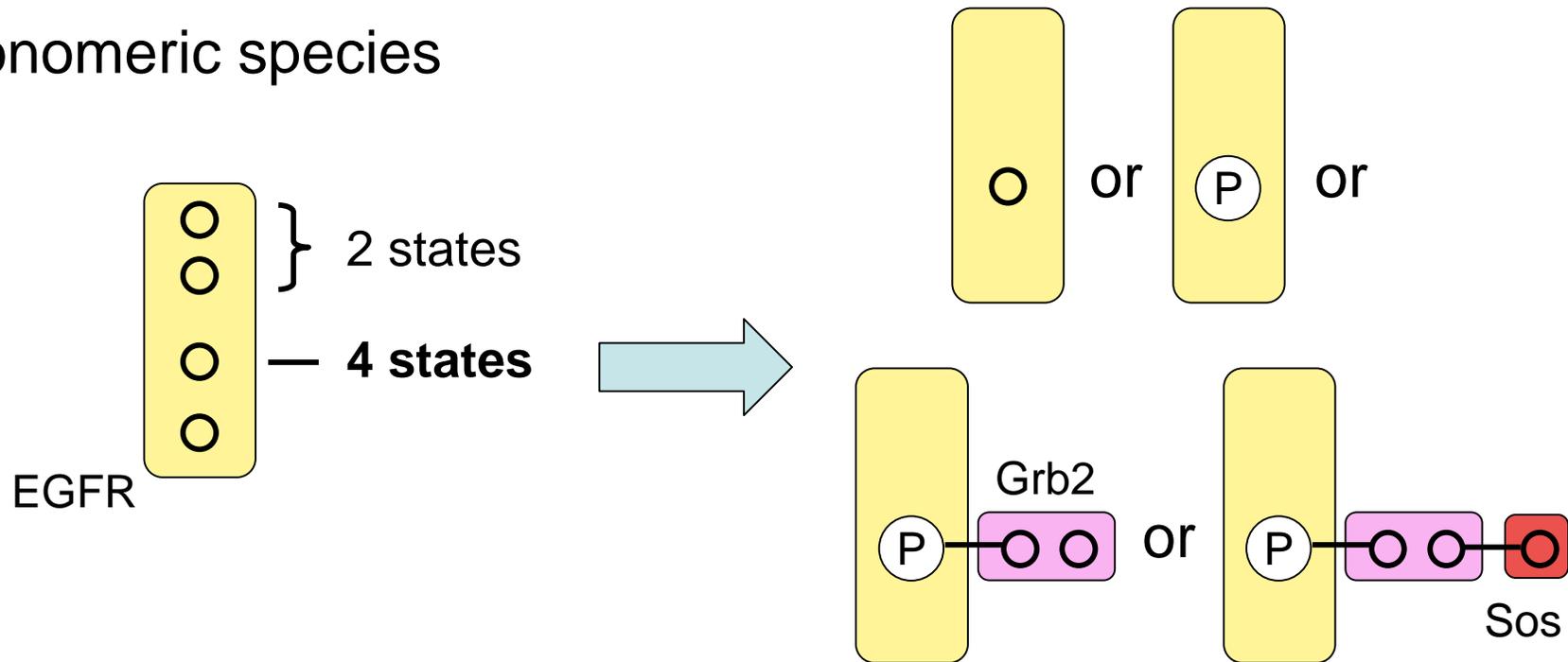
Combinatorial complexity of early events

Monomeric species



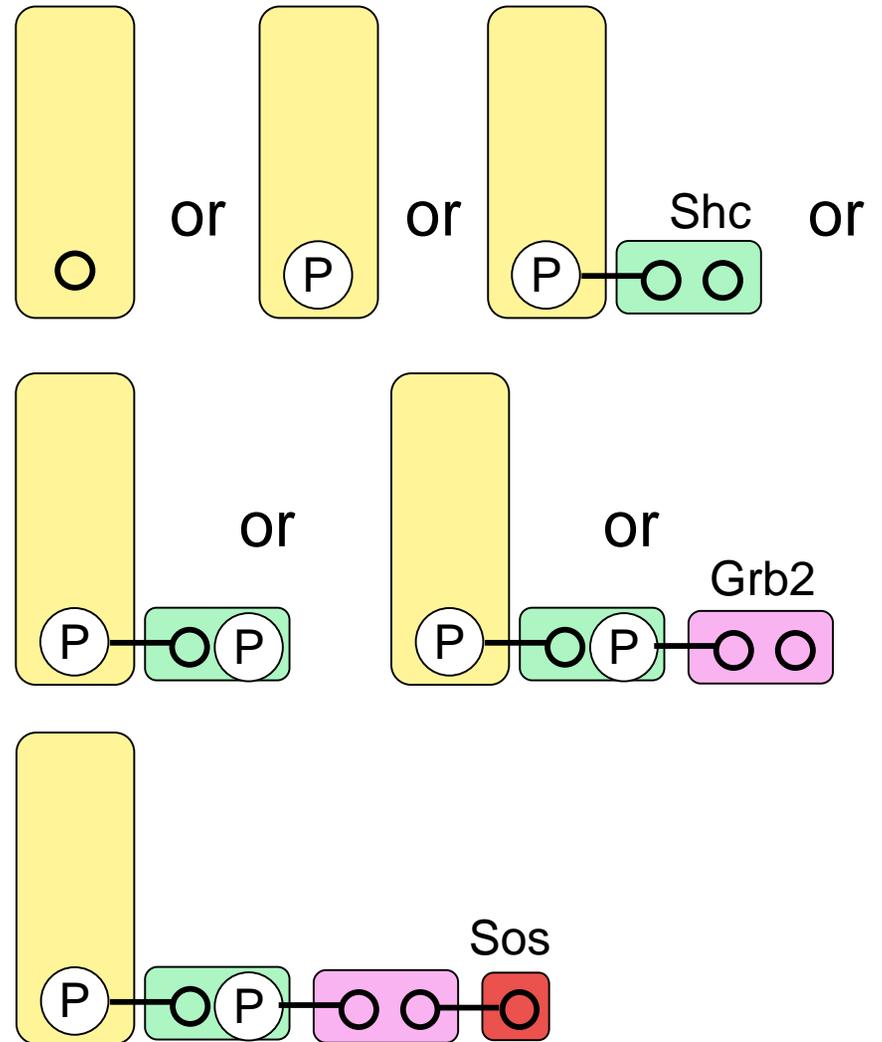
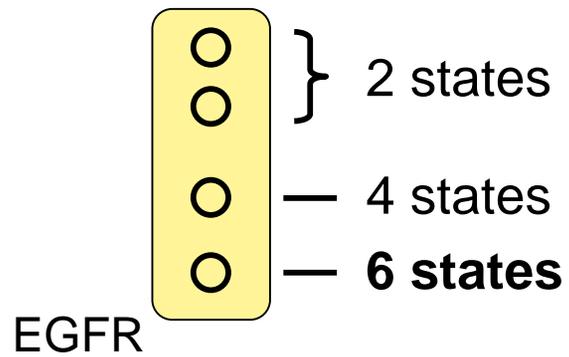
Combinatorial complexity of early events

Monomeric species

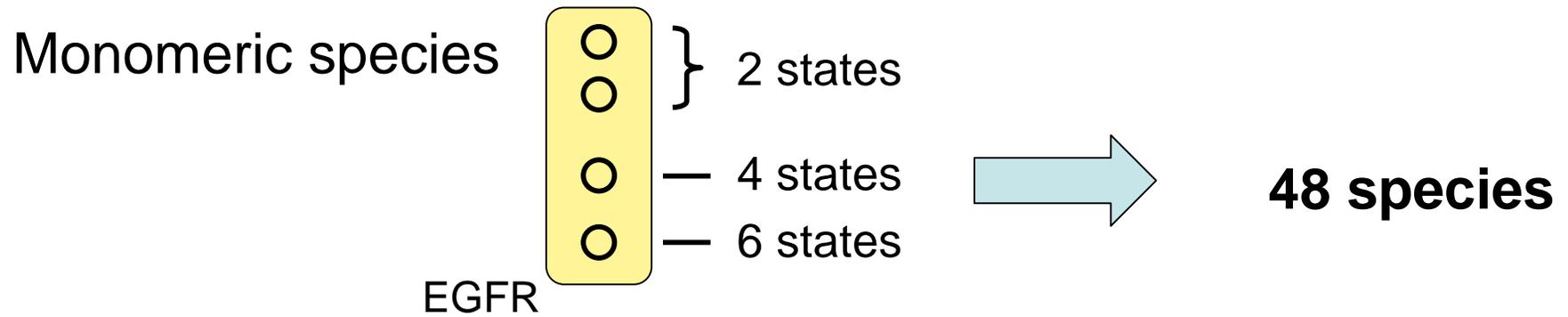


Combinatorial complexity of early events

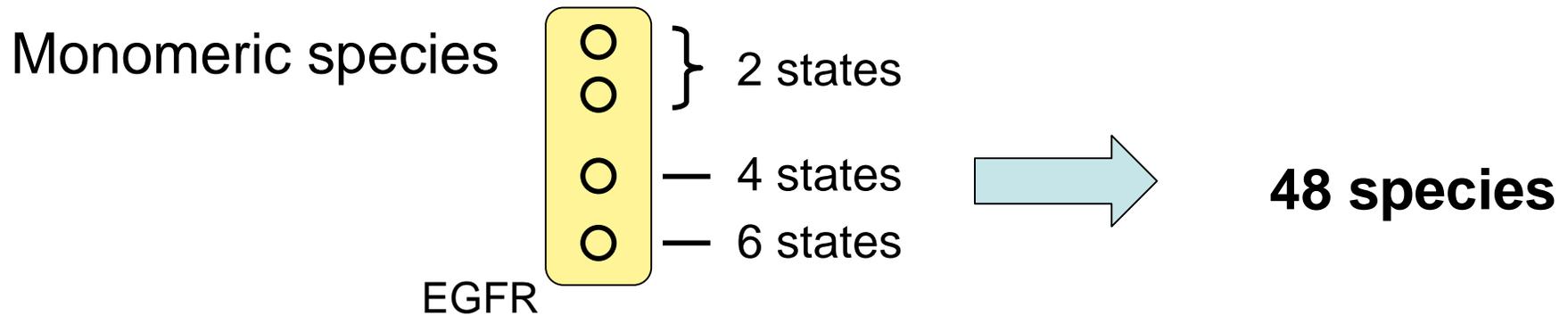
Monomeric species



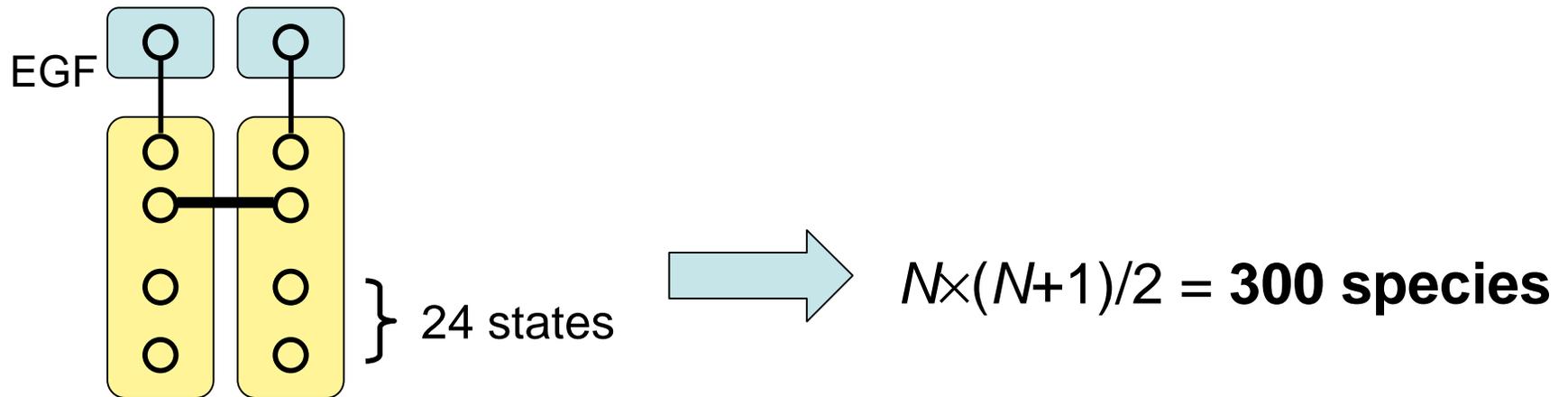
Combinatorial complexity of early events



Combinatorial complexity of early events



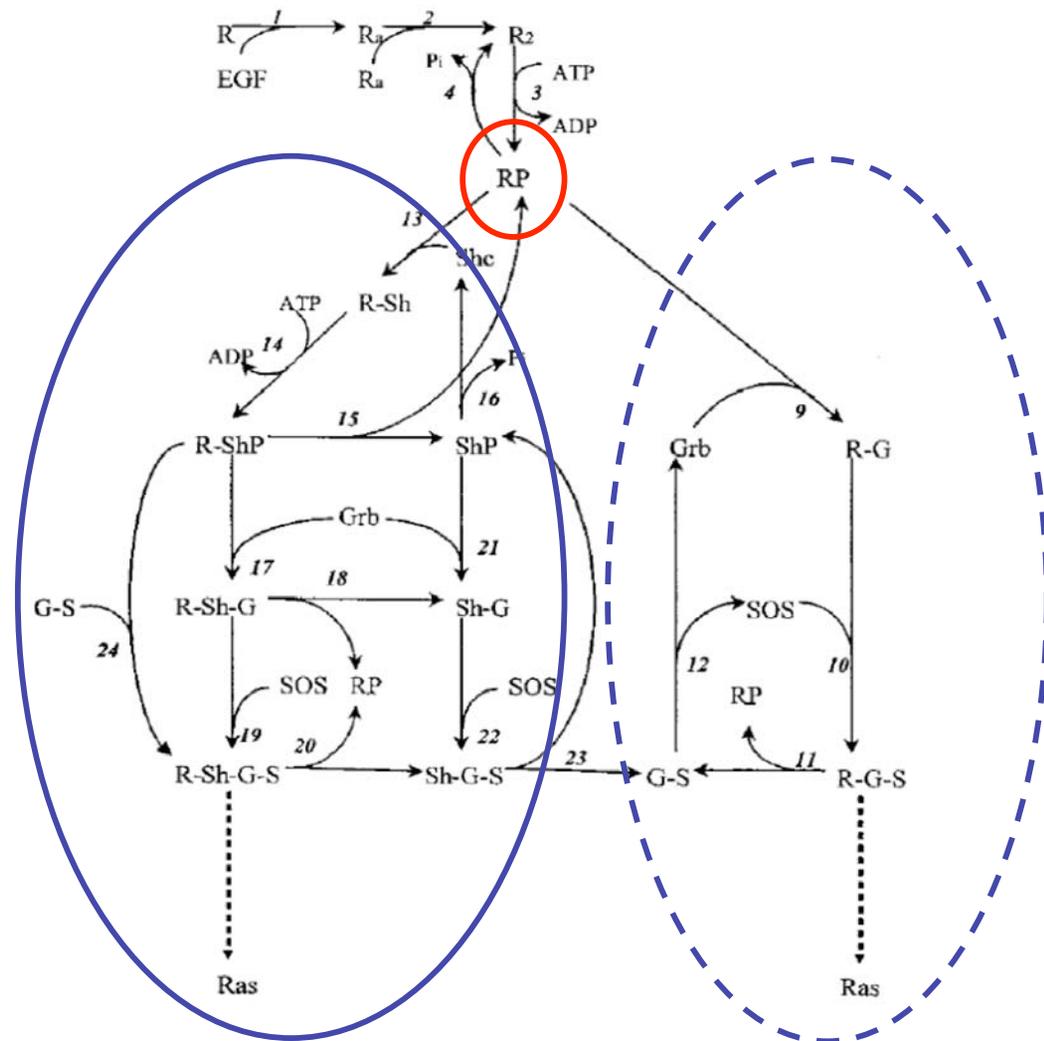
Dimeric species



Assumptions made to limit combinatorial complexity

1. Phosphorylation inhibits dimer breakup
2. Adaptor binding is competitive

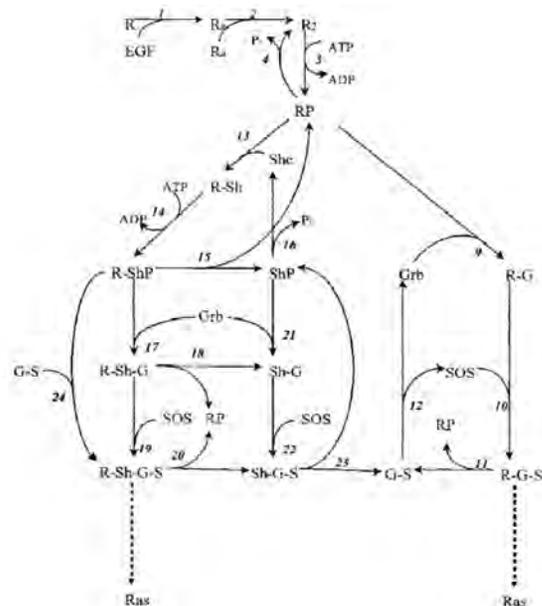
Experimental evidence contradicts both assumptions.



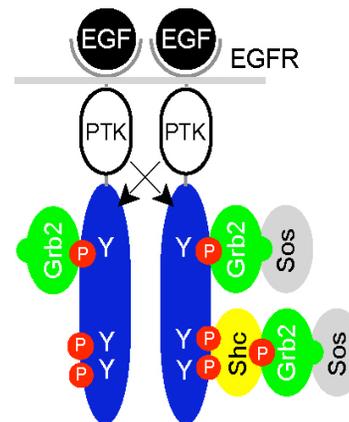
Rule-based version of the Kholodenko model

- 5 molecule types
- 23 reaction rules
- No new rate parameters (!)

18 species
34 reactions



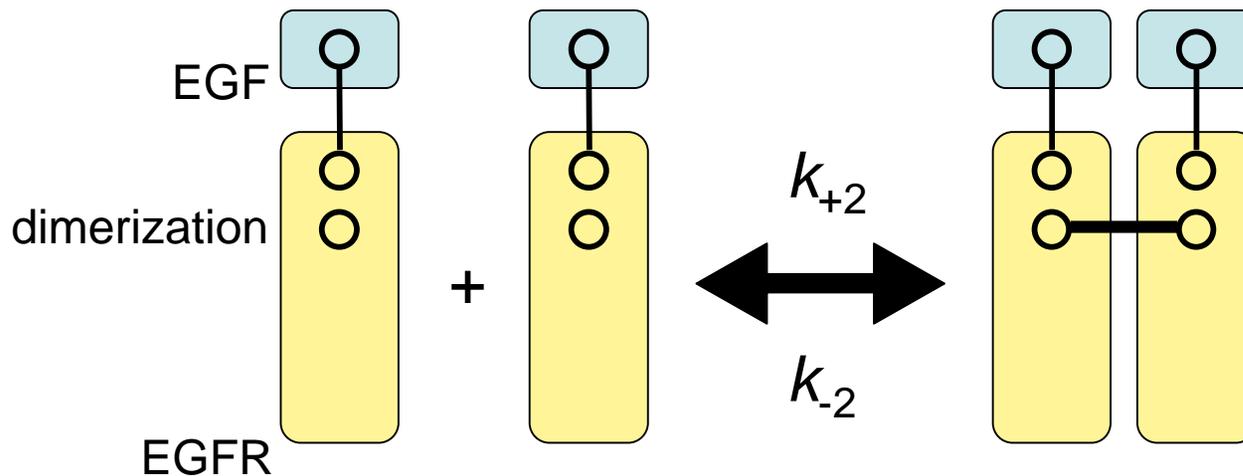
356 species
3749 reactions



Blinov et al. *Biosystems* **83**, 136 (2006).

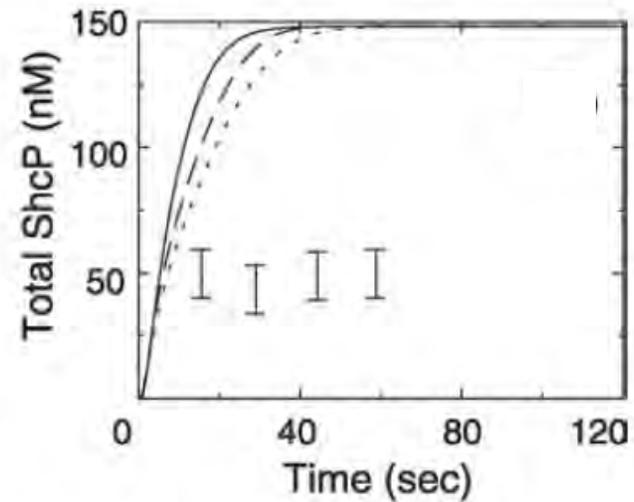
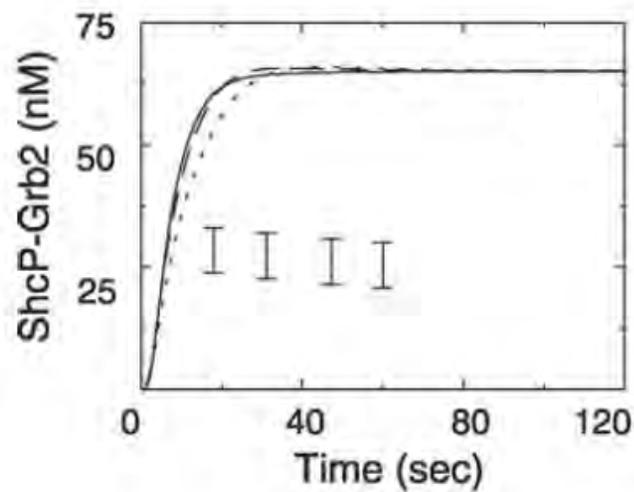
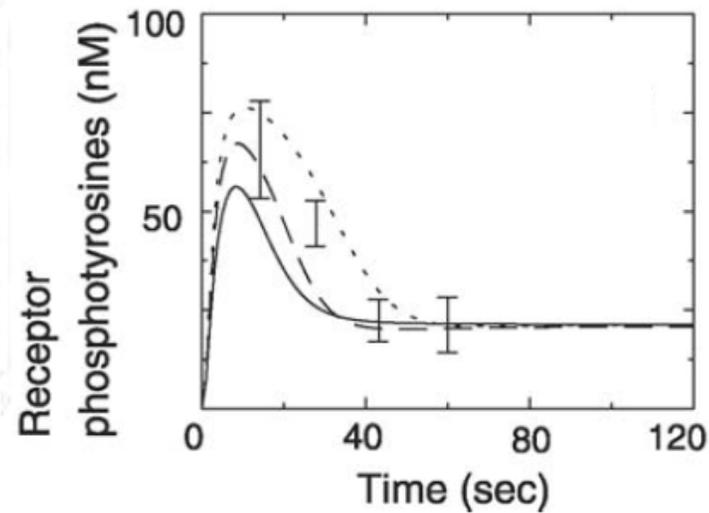
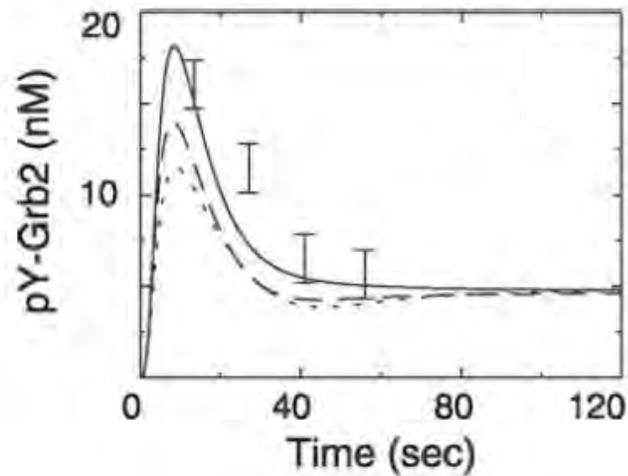
Dimerization rule eliminates previous assumption

EGFR dimerizes (600 reactions)

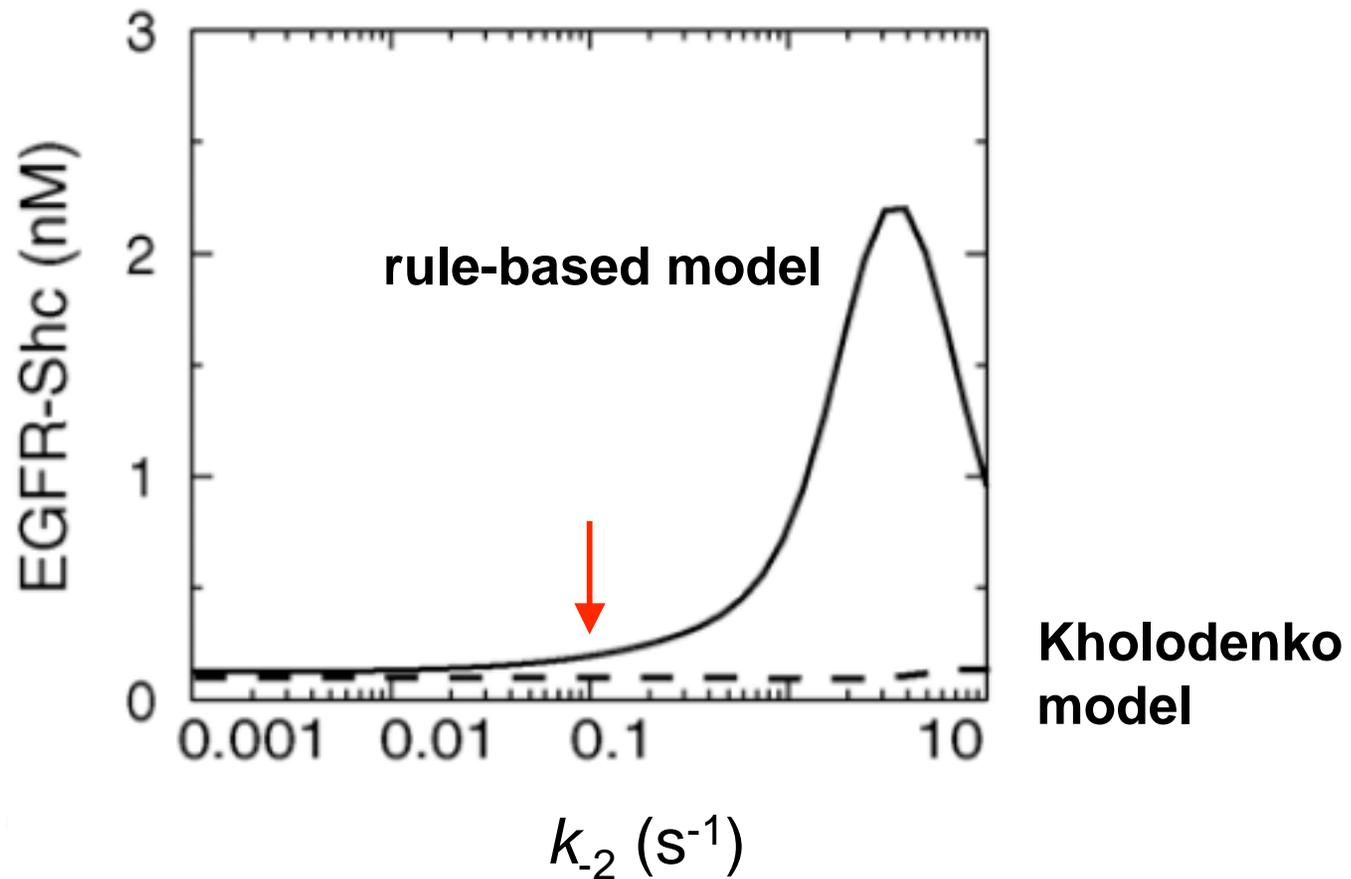


Dimers form and break up independent of phosphorylation of cytoplasmic domains

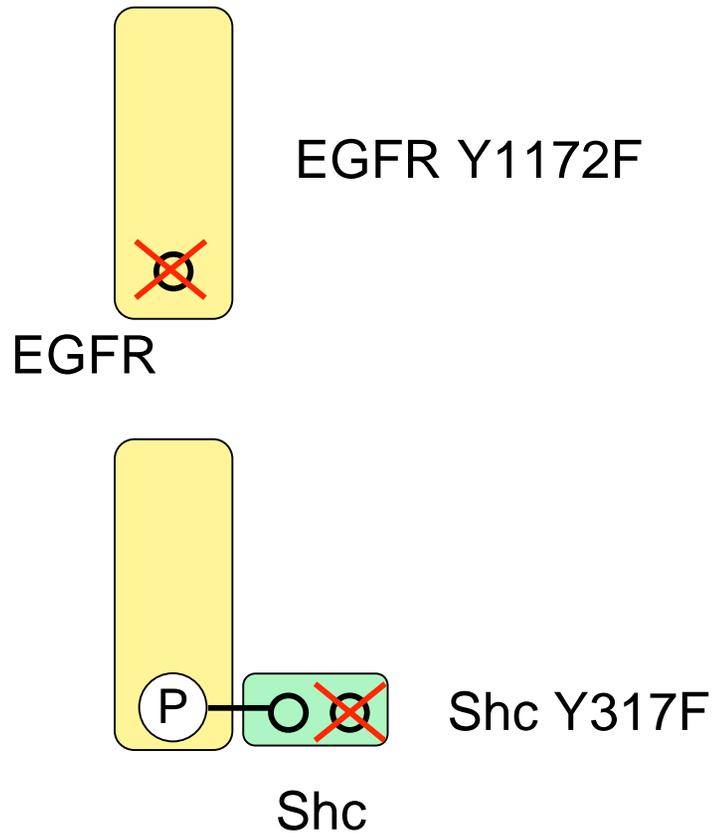
Two models predict similar overall binding and phosphorylation kinetics



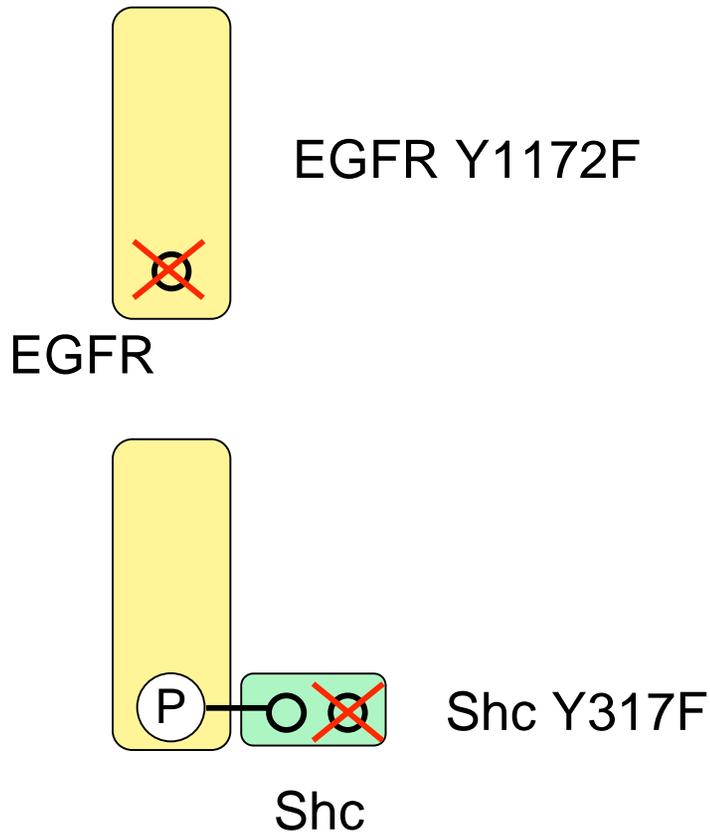
Strong differences when dimer dissociation rate is varied



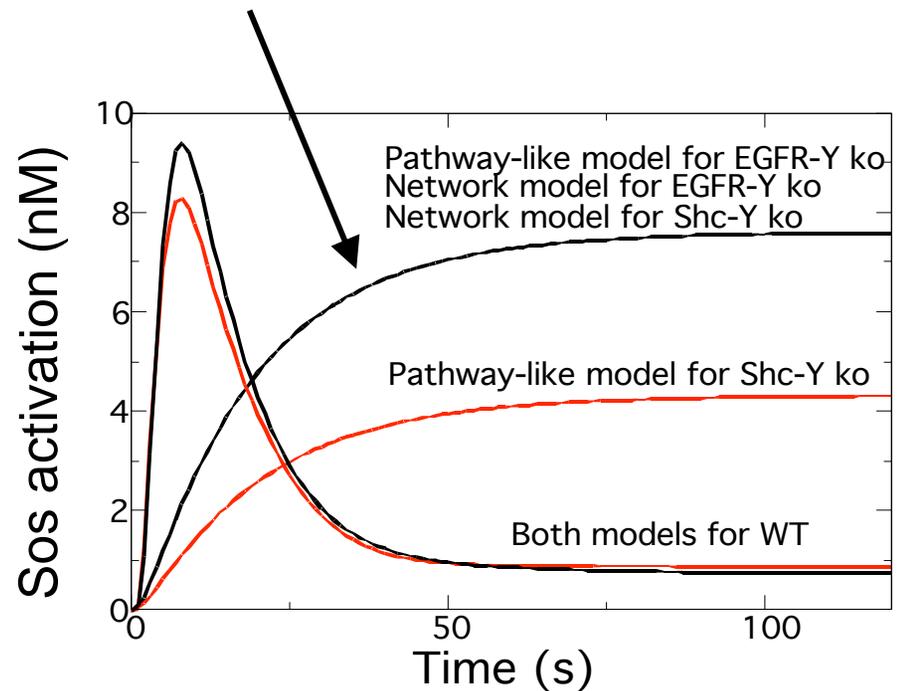
Results for two different knockouts of the Shc pathway



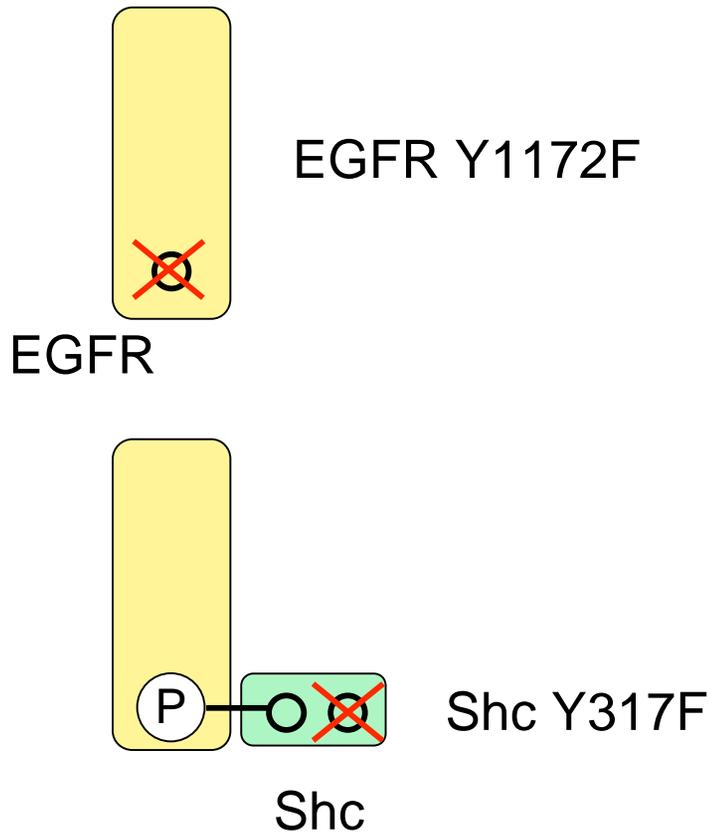
Results for two different knockouts of the Shc pathway



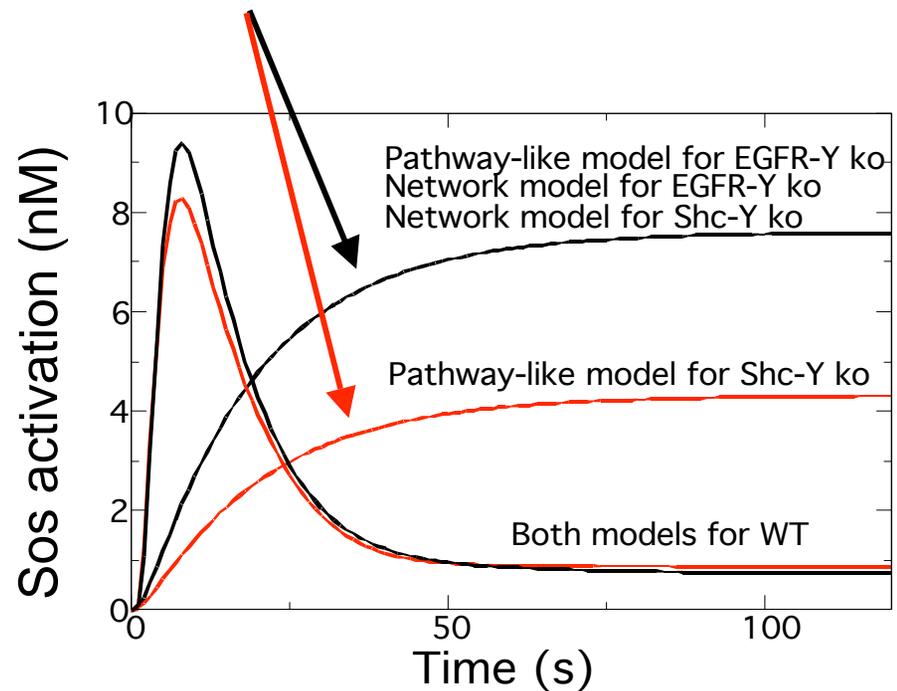
Rule-based model predicts same behavior for both knockouts



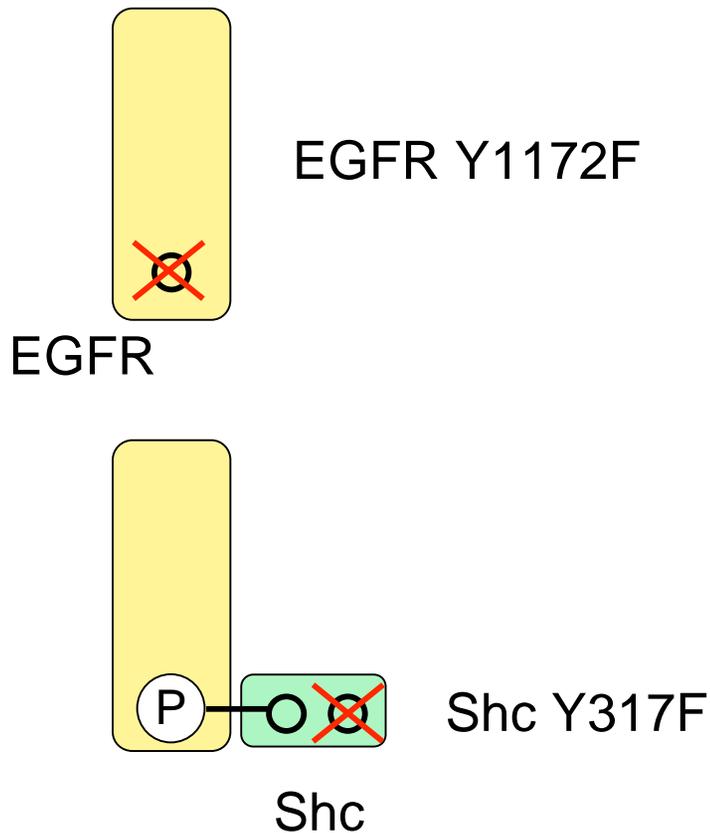
Results for two different knockouts of the Shc pathway



Kholodenko model predicts lower activation for **Shc Y317F**



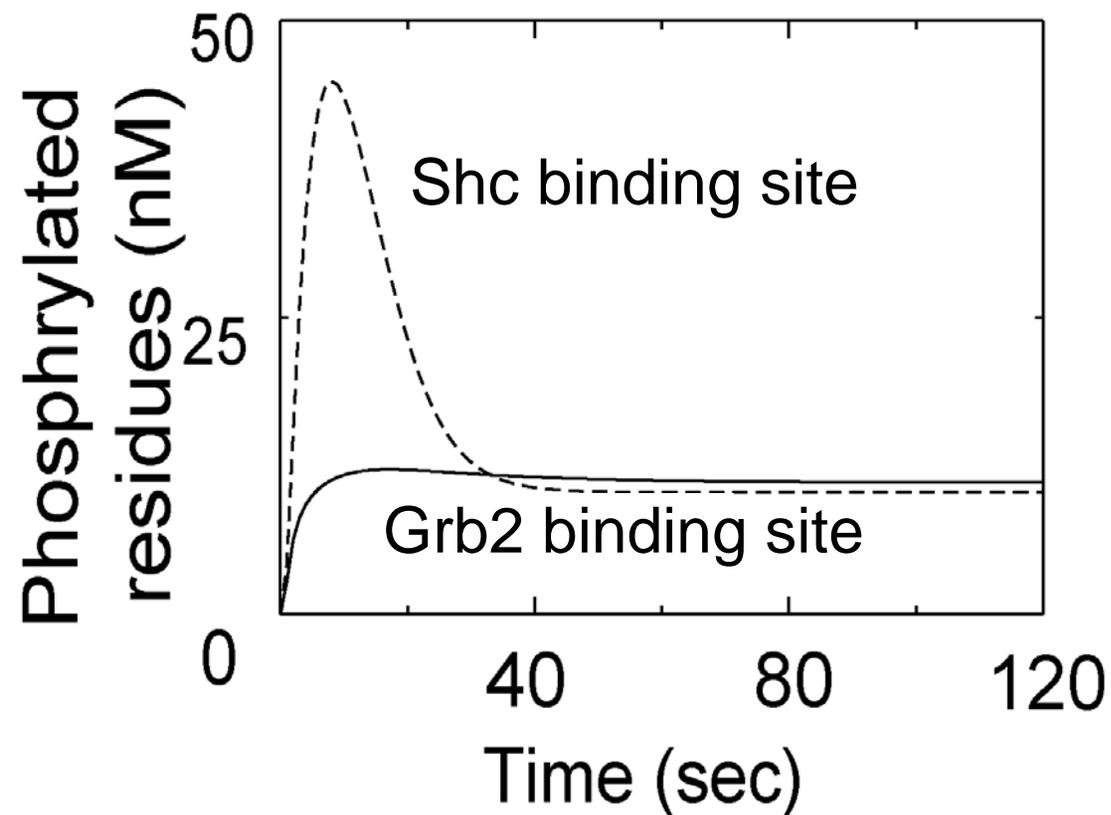
Results for two different knockouts of the Shc pathway



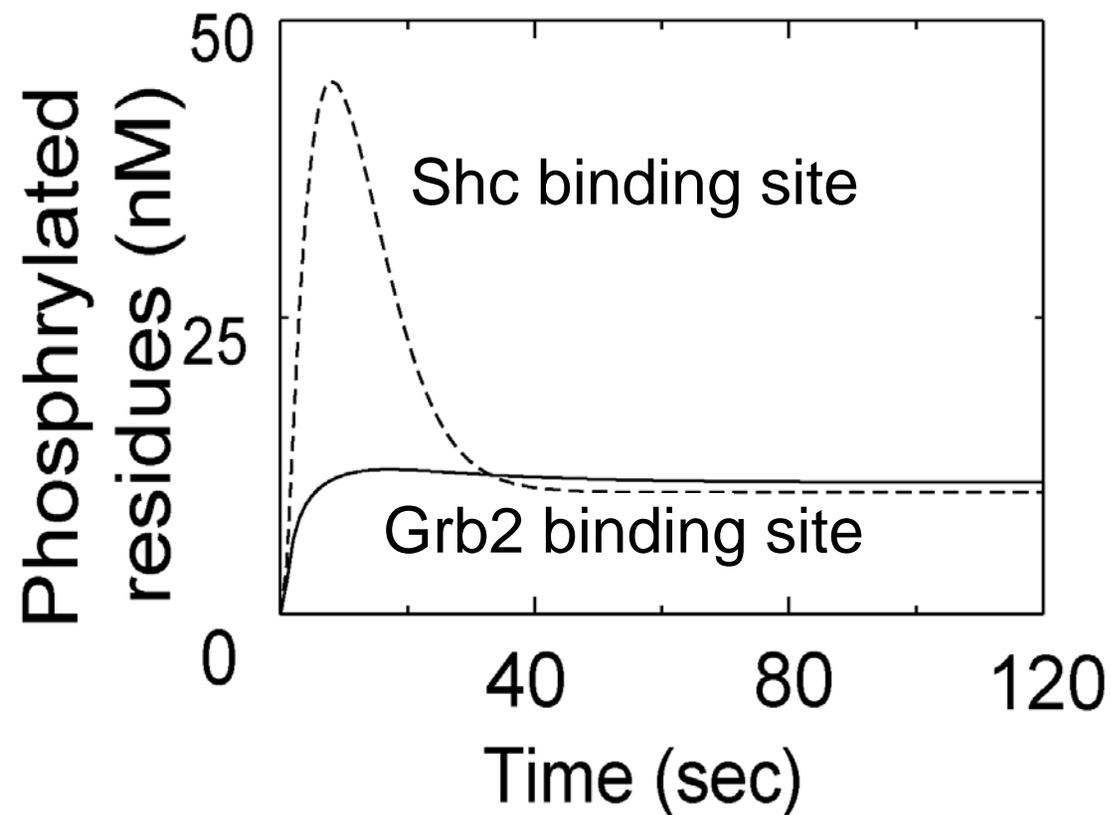
Kholodenko model
predicts lower activation
for **Shc Y317F**

... because mutant Shc
blocks binding of Grb2
(competitive binding)

Rule-based model predicts distinct kinetics for two phosphorylation sites

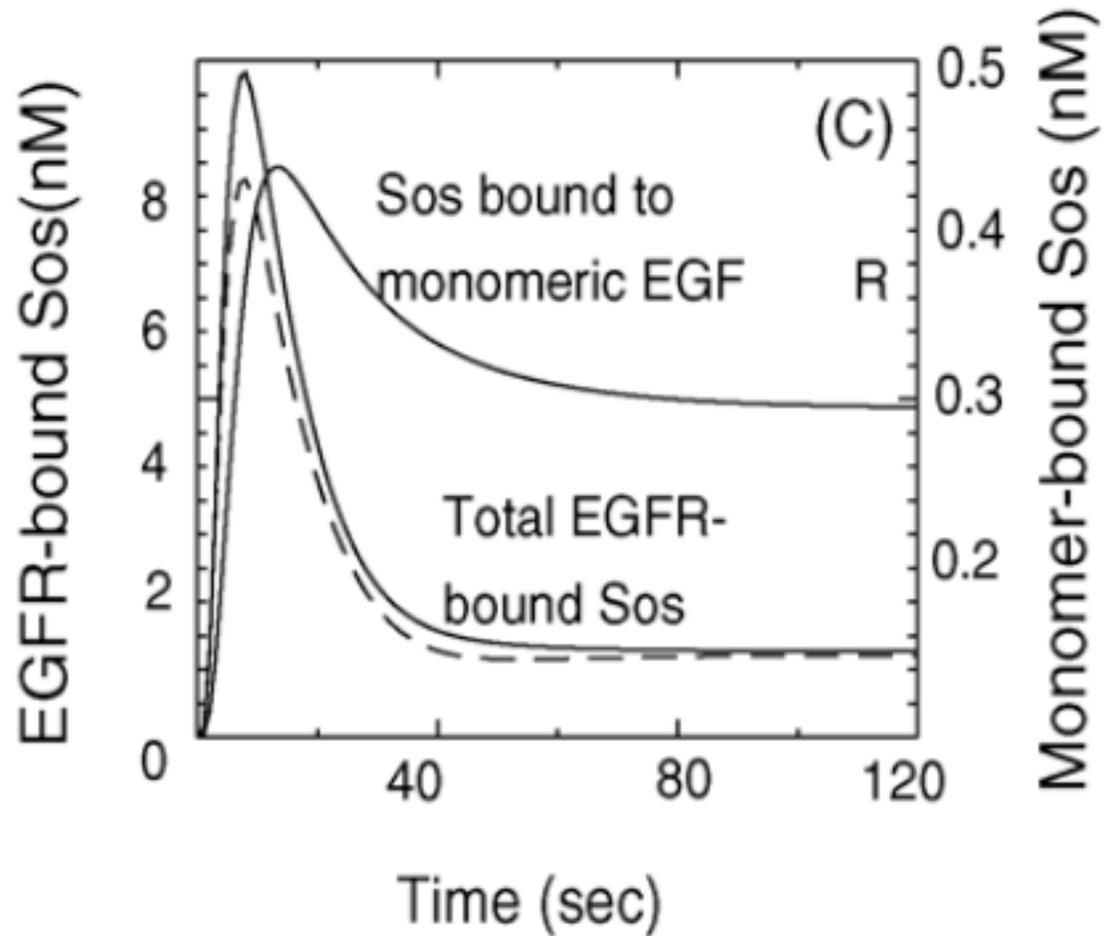
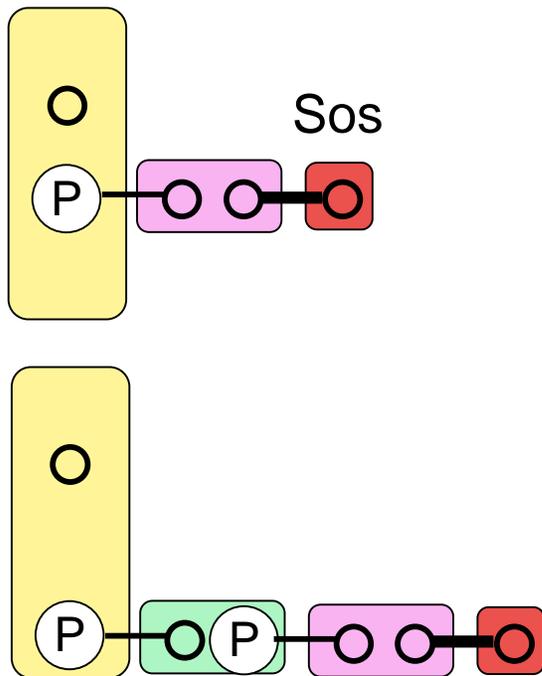


Rule-based model predicts distinct kinetics for two phosphorylation sites

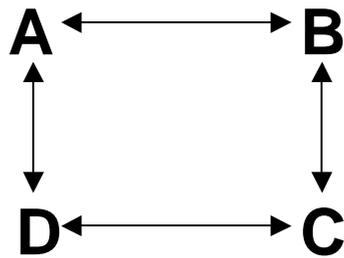


Also predicts monomers make substantial contribution to steady state Sos activation

36% of active Sos associates with EGFR monomers



Principle of detailed balance: Making sure that models obey laws of thermodynamics



Around any loop in the reaction network, the total free energy change (ΔG) must equal 0.

$$\Delta G = \Delta G_{AB} + \Delta G_{BC} - \Delta G_{DC} - \Delta G_{AD} = 0$$

$$\Rightarrow -RT(\ln K_{AB} + \ln K_{BC} - \ln K_{DC} - \ln K_{AD}) = 0$$

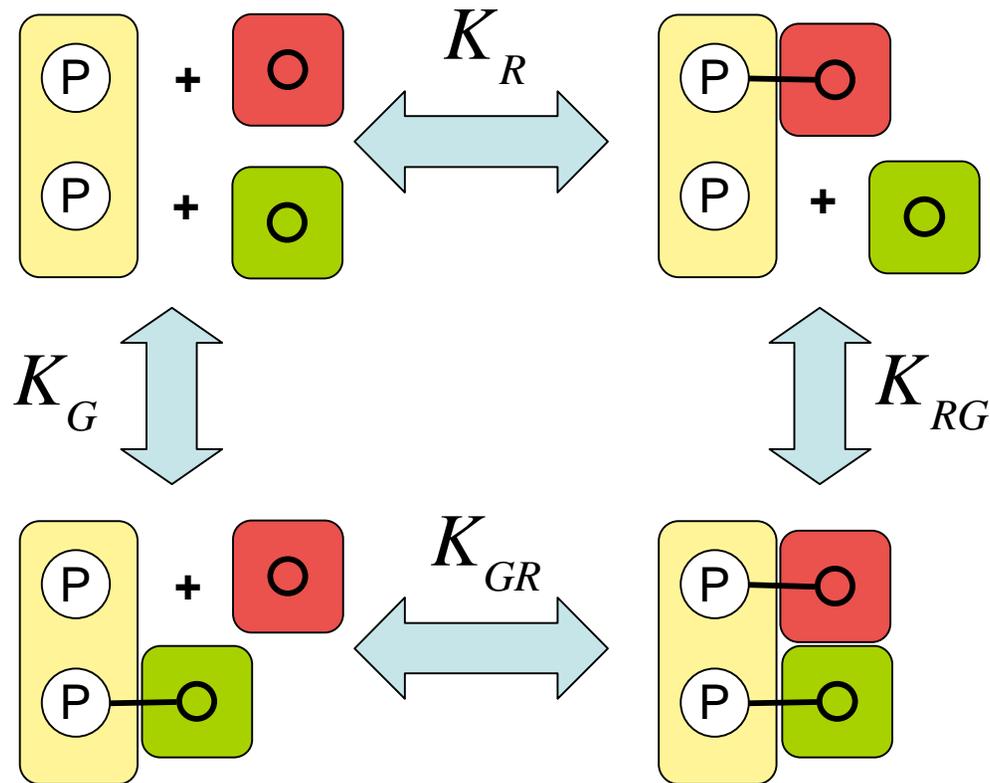
$$\Rightarrow K_{AB} K_{BC} / K_{DC} K_{AD} = 1$$

Kholodenko model has 5 such constraints, but some subsequent models have not enforced these.

See reference list on the q-bio wiki (Lecture 2, Bibliography and Links).

Worked example: cooperative binding to a scaffold

Xmas chile scaffold (XCeS) protein



$$K_R K_{RG} = K_G K_{GR}$$

“The enchilada is just as hot no matter which chile you eat first.”

...but where's the **SMOKING GUN**?

Question is often raised: “Does the data available justify this complicated approach?”

We can argue with the question, but we are still looking for the definitive application where RBM is absolutely required and provides novel insight.

q-bio Model Inspection Program (aka Project 3)

“Looking for (Models of Mass Deception)” (MMD)

Suspicious assumptions to look for (and test)

- *Sequential activation*
 - Particularly analyses whose results depend on such assumptions
- *Exclusive (one-at-a-time) interactions* or limits on the stoichiometry of complexes
- *Violations of principle of detailed balance*
 - Check model of Schoeberl et al. (*Nat. Biotechnol.*, 2002)

cellsignaling.lanl.gov

Michael Blinov
Jin Yang
Ambarish Nag
Michael Monine
Fangping Mu

Matthew Fricke
Leigh Fanning
Nathan Lemons
James Cavanaugh
Jeremy Kozdon
Paul Loriaux
Michelle Costa
Agate Ponder-Sutton
Michael Saelim
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